Avian Diseases

NOVEMBER 1957

Case report—Lens opacities in fowls possibly associated with epidemic tremors ..... M. C. PECKHAM 247

The pathology of duck virus hepatitis
JULIUS FABRICANT, C. G. RICHARD AND P.P. LEVINE 256

Oral therapy for erysipelas in turkeys
CLYDE I. BOYER JR., AND J. A. BROWN 275

Selected neoplasms in exotic birds
JAMES R. WADSWORTH AND W. C. OSMAN HILL 290

Salmonella heidelberg infection as a problem in pullorum disease control
W. E. BURR, M. TOURTELLOTE, R. E. LUGINBUHL, AND E. L. JUNGHERR 298

A study on the criteria for evaluating infectious bronchitis immunity
P. W. CHANG, A. H. DARDIRI, DOROTHY E. FRY AND VANCE J. YATES 304

The distribution of the infectious synovitis agent in the tissues of artificially infected chickens ..... M. S. COVER AND W. J. BENTON 312

The increased incidence of visceral lymphomatosis in broiler and replacement birds ..... W. J. BENTON AND M. S. COVER 320

The influence of furazolidone on experimental and natural pullorum infection in the chicken ..... C. F. SMYSER, JR. AND H. VAN ROEKEL 328

Further studies on pullorum disease in turkeys
M. L. WRIGHT, G. W. ANDERSON, N. A. EPPS, AND R. B. TRUSCOTT 338

Abstracts of papers presented at the 29th northeastern conference on avian diseases (formerly northeastern conference of laboratory workers in pullorum disease control) June 24-26, 1957, Pennsylvania State University, University Park, Pa. 345

Index Volume 1 350

Avian Diseases

VOL. I: No. 3, NOVEMBER 1957

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CASE REPORT—LENS OPACITIES IN FOWLS POSSIBLY ASSOCIATED WITH EPIDEMIC TREMORS*

By M. C. PECKHAM

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OCULAR lesions characterized solely by grayish opacities of the lens have been seen in three flocks of White Leghorns and one flock of cross-bred birds (accessions 56565, 56624, 56636, 56830, 56880, 57098) in which two to three percent of the birds were affected. The birds were three to four months of age when the owners first noticed that some of the birds acted blind or had difficulty in walking.

Either the entire lens appeared to be covered with a gray, filmy network or a linear, grayish opacity was visible in part of the lens (figures 1, 2). The condition was either bilateral or unilateral. The birds did not exhibit photophobia and there was no lacrimation or accumulation of exudate beneath the nictitating membrane. On exposure to light, the pupillary reflex was elicited. The iris was of normal color and the pupil was regular in outline. The fluid in the anterior chamber was clear. When enucleated, the eyeball was of normal size and shape.

In most cases, the birds with eye lesions also exhibited nervous symptoms. Some birds acted blind and walked very cautiously. One bird had a stilted walk and a tremor of the

* Presented at the 29th Northeastern Conference on Avian Diseases (formerly the Annual Meeting of the Northeastern Conference of Laboratory Workers in Pullorum Disease Control) June 24, 1957, Pennsylvania State University, University Park, Pa.

head and neck. Other birds rested on their hocks and were reluctant to move. One bird while standing, made clutching movements with its toes and had a constant quivering of its tail as though trying to maintain its balance. When walking, this bird raised its legs high with a jerky movement. One affected chicken, held for observation for five weeks, showed no improvement.

The histories of the four flocks affected with this condition indicated a higher than normal early chick mortality. In two cases, a diagnosis of epidemic tremors had been made at this laboratory when the birds were two weeks of age. At that time confirming evidence for this diagnosis was the history, clinical symptoms and histological findings in the brain and viscera. In a third case, the owner reported ataxia in four percent of the flock during the first few weeks. No laboratory diagnosis had been procured. A fourth case had suffered heavy losses as chicks and had been diagnosed as pantothenic acid deficiency by another laboratory. None of these cases had any history of respiratory disease and the hemagglutination-inhibition test for Newcastle disease was negative.

Histological findings. All tissues for histological examination were collected from freshly killed birds, fixed in ten percent aqueous formalin and stained with hematoxylin and eosin. Dr. L. Z. Saunders of the Brookhaven National Laboratory reported on one eye section. He found that the lens had a cataract formation which was particularly well marked at the lateral and medial edges and in the posterior subcapsular zone with extension into the center (figure 3). There was proliferation of the capsular epithelium and at the peripheries, some of the proliferating epithelium was invaded by fibroblasts (figure 4). There were areas of synechia between the anterior capsule of the lens and the pigment epithelium of the iris. Mononuclear infiltration was present in the iris. No changes were noted in the sclera or its cartilages. No inflammatory lesions were seen in the retina.

Dr. T. C. Jones of the Armed Forces Institute of Pathology, reported on another specimen. He found there was a

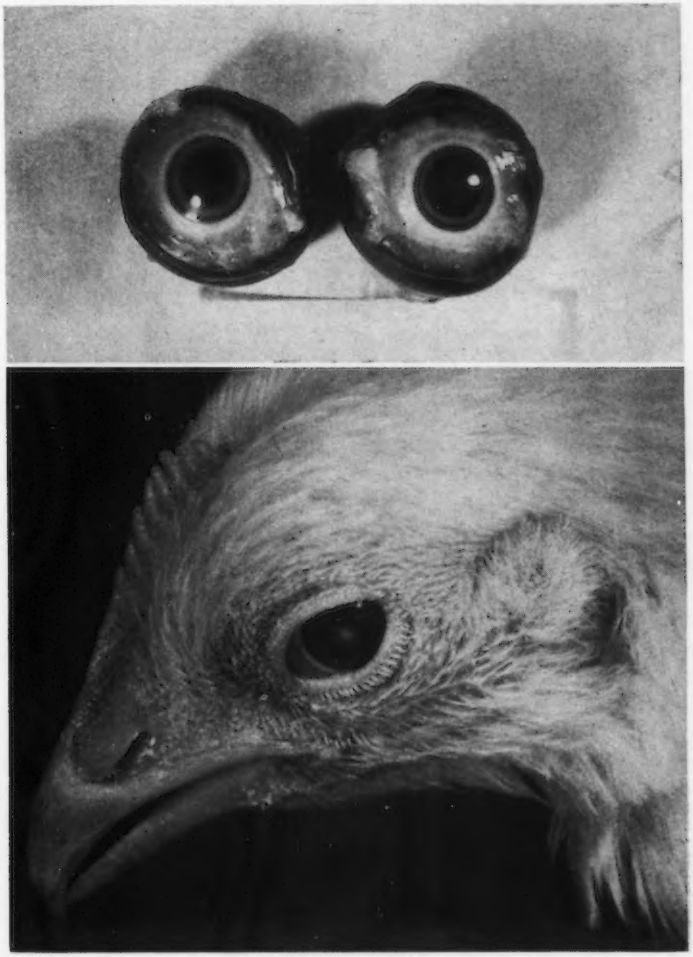

Fig. 1. (top) Normal eye on left. Cataractous lens in eye on right.

Fig. 2. (bottom) Twelve-week-old chicken with a diffuse opacity of the lens.

loss of lens protein and collapse of the capsule. The iris and ciliary body were infiltrated with lymphocytes and numerous plasma cells. The filtration angles were patent. No changes were seen in the retina or optic nerve. The brain from this bird had neuronophagia and satellitosis in the medulla and corpus striatum. Centrally in the white matter of the cerebellum, a number of deeply stained neurons were observed in each of which one, large, eosinophilic, spherical mass was present within the nucleus. Within the molecular layer of the cerebellum, an occasional focus of gliosis was noted. Endothelial hyperplasia of small blood vessels occurred occasionally.

Examination of other brains from affected birds revealed gliosis, perivascular cuffing and endothelial hyperplasia (figures 5, 6). An occasional perivascular cuff was noted in the lumbar region of the spinal cord. Lymphoid foci were found in the kidney, liver, pancreas, heart and proventriculus.

Cultural studies. Standard bacteriological techniques were used in attempts to isolate an agent from the ocular fluid and visceral organs. No bacterial or fungal agent was isolated. Brains, ocular fluid and livers were harvested aseptically from affected birds and inoculated into the yolk-sac and chorio-allantoic cavity of embryonating eggs for three serial passages. Ocular fluids and brain suspensions were inoculated intra-ocularly into 11-day embryos for five serial passages in an effort to cultivate the virus of epidemic tremors. No mortality or morphological changes were produced in the embryos. It should be mentioned that eggs from the same flock were later found unsuitable for the cultivation of the epidemic tremors virus. Inoculaton of ocular fluid from affected eyes into the anterior chamber of the eyes of normal chicks gave negative results.

DISCUSSION

This condition, on gross examination, appeared to resemble that described by Feibel[7,8] in his work with epidemic

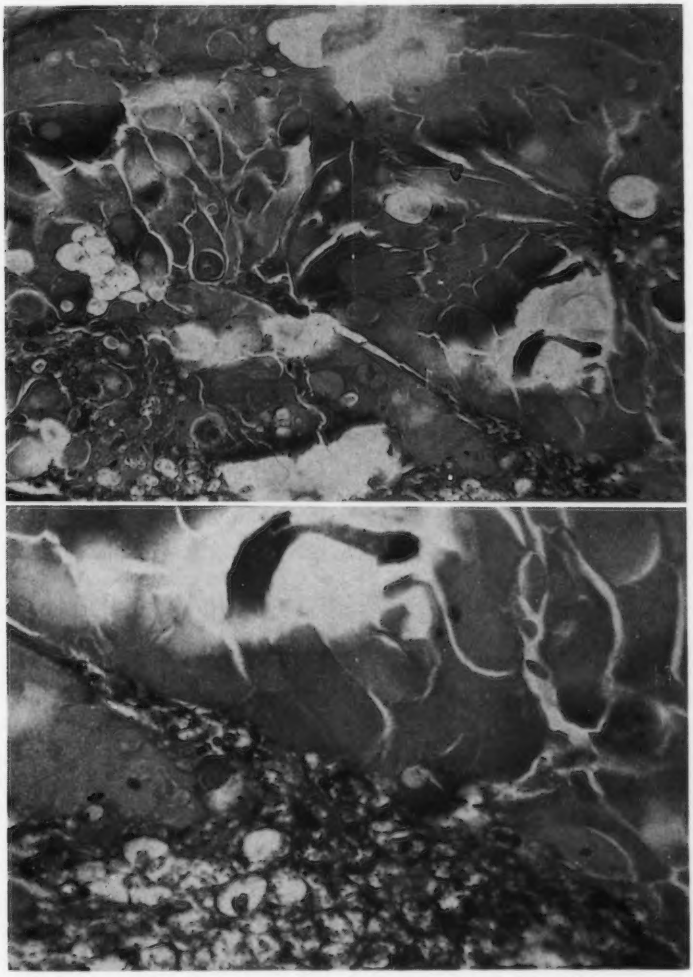

Fig. 3. (top) Cataractous lens with mosaic pattern caused by fragmentation and vacuolation of lens protein. There is invasion of lens protein by inflammatory cells. (low power)

Fig 4. (bottom) Cataractous lens with proliferation of capsular epithelium. (high power)

tremors. By intraocular inoculation with epidemic tremors virus, he produced, "a rectangular silver-colored patch (probably due to involvement of the lens)." Eight out of thirty showed these gross lesions. "On histological study, 16 out of 30 showed definite to marked infiltration by mononuclear cells (iridocyclitis) of the iris and frequently the ciliary body, although nine of these eyes appeared grossly normal. Brain lesions were typical of AE in chicks while controls were negative."

It should be noted that the birds we examined at three to four months of age had been affected with epidemic tremors or a condition similar to epidemic tremors as young chicks. Lesions in the brain sections from the two week old chicks and from the older birds were comparable with those generally described for epidemic tremors.

Zander[16] has also observed blindness in birds following an outbreak of epidemic tremors. The gross and microscopic lesions he found in the eye are similar to the lesions in our cases. He also succeeded in producing symptoms of epidemic tremors in chicks by intra-cerebral inoculation of ocular fluid taken from affected eyes.

Ocular lesions due to bacterial infections have been reported by various workers.[1,6,9,10,11] Where a gross description has been given, it has differed from the condition described herein. All our attempts at isolation of bacteria were negative. Ocular lesions ascribed to fungus infection have been reported by several workers.[12,13,14] In these cases the diagnosis was confirmed by isolation of the fungus. We have been unable to demonstrate a fungus by cultural methods or histological examination.

Brandly[4], in discussing Newcastle disease, mentioned a clouding or graying of the eye resulting from the suspension of inflammatory cells in the fluid of the anterior chamber. Ocular lesions in our birds differed in that the fluid in the anterior chamber was clear.

Bullis[5] described a keratoconjunctivitis characterized by an erosion of the cornea. This was caused by ammonia fumes.

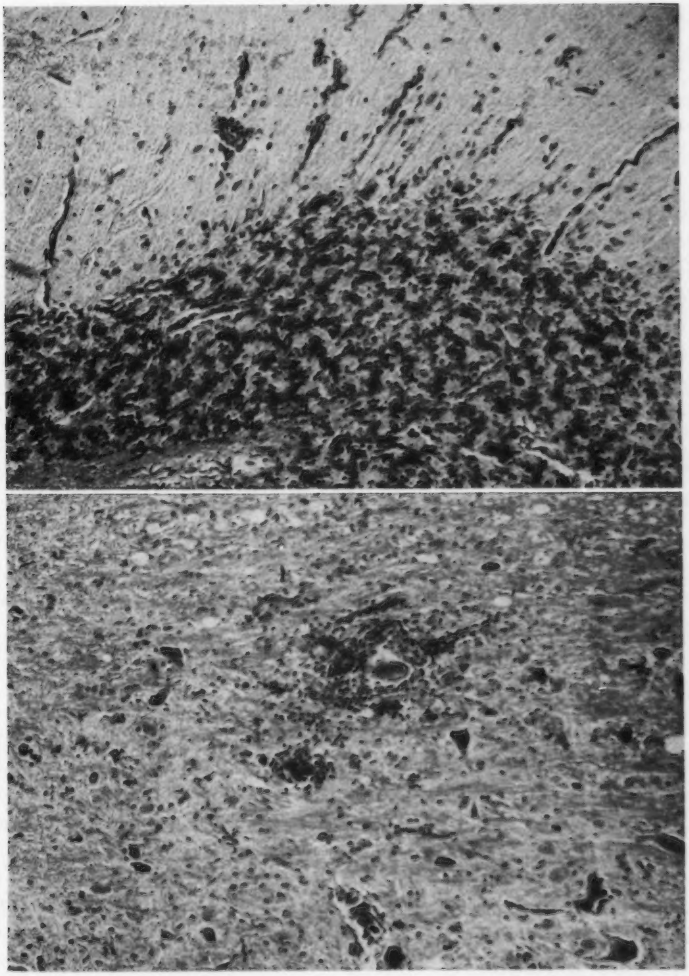

Fig. 5. (top) Cerebellum with endothelial proliferation in the molecular layer. (low power)

Fig. 6. (bottom) Brain section with perivascular cuffing and degenerated neuron in center of photograph. (low power)

Affected birds exhibited photophobia. In our case there was no lesion of the cornea and no photophobia. Biering-Sorenson[3] described chorioretinitis in hens associated with toxoplasmosis. Since the lesions in our birds were primarily an iridocyclitis with cataract formation, there would appear to be no relation between the two conditions.

Saunders and Moore[15] described a blindness in turkeys due to granulomatous chorioretinitis of unknown eitology. In most cases the cornea was opaque or else the lens was cataractous. Grossly (there was a collapse of the cornea and decrease in the anteroposterior dimension. Although the lenses in our chickens were cataractous, the cornea was never opaque and there was no shrinkage of the eyeball. Bierer[2] described keratoconjunctivitis in turkeys which was characterized by considerable exudate under the lids. Its cause is unknown, but it is quite different from the condition described in this paper since we found no conjunctivitis and no involvement of the cornea.

ACKNOWLEDGMENTS

I wish to express my appreciation to Dr. L. Z. Saunders of the Brookhaven National Laboratory for the photomicrographs used in this paper.

SUMMARY

Ocular lesions which occurred after infections with epidemic tremors have been seen in three flocks of chickens three to four months old. The eyes had a diffuse or linear opacity of the lens. Histologically, there was cataract formation and iridocyclitis. No organisms were isolated nor were any seen in tissue sections. Since a similar condition has been produced experimentally with the virus of epidemic tremors by other workers, it is suggested that the condition described herein may represent a gross, morphological expression of epidemic tremors.

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THE PATHOLOGY OF DUCK VIRUS HEPATITIS

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Received 25 Sept 1957

IN 1950 Levine and Fabricant[6] described a new disease of young ducklings characterized by high mortality and rapid death. The significant lesions associated with the disease were an enlarged liver, containing necrotic areas of different sizes and numerous hemorrhagic spots ranging from petechiae to ecchymoses. The disease was demonstrated to be caused by a virus which was readily cultivated in chicken or duck embryonating eggs. A practical and successful method of controlling the disease was instituted. Specific antiserum collected from ducks recovered from the natural disease protected exposed ducklings when they were injected at the onset of an outbreak.

Although the disease was first seen in the Long Island duck producing area in 1949, an outbreak of a similar disease was seen by Levine and Hofstad[7] in Western New York as early as 1944. The disease has since been diagnosed in Massachusetts, Illinois,[4] and Michigan in the United States as well as in England,[1,2] Canada,[8] Germany,[9] Egypt[9] and the Netherlands.[10] In 1955, a preliminary report[3] on the pathology of this disease was presented. This paper presents a more complete account of the pathology and pathogenesis of duck virus hepatitis.

SEROLOGY

As was noted in previous papers,[5,6] serum-neutralization tests in embryonating chicken eggs gave no indication that duck hepatitis virus was related to either Newcastle virus or to the duck plague virus of Jansen and Kunst. A series of neutralization tests with normal and convalescent sera from cases of canine hepatitis and human infectious hepatitis revealed no serologic relationship to these agents. The canine hepatitis sera were secured through the courtesy of Dr. J. H. Gillespie of the Veterinary Virus Research Laboratory of Cornell University. The normal human sera were secured from Dr. H. W. Ferris of the Tompkins County Laboratory and the convalescent infectious hepatitis sera from Dr. J. R. Paul of Yale University, School of Medicine.

TABLE I

Sample protocol of duck hepatitis virus-neutralization test conducted with canine hepatitis immune serum and normal canine serum in embryonated chicken eggs.

	Virus dilution (reciprocal of log)					
	1	*2*	*3*	*4*	*5*	*6*
Virus and Broth	—	8/8	8/8	8/8	5/8	0/8
Virus and Duck Hepatitis Immune Serum	6/8	6/8	3/8	0/8	0/8	—
Virus and Normal Dog Serum	8/8	8/8	8/8	5/8	2/8	—
Virus and Canine Hepatitis Immune Serum	8/8	8/8	8/8	5/8	6/8	—

Numerator = number embryos with lesions
Denominator = number embryos infected

TABLE II

Sample protocol of duck hepatitis virus neutralization test conducted with human infectious hepatitis immune serum and normal serum in chicken embryos.

	Virus dilution (reciprocal of log)					
	1	*2*	*3*	*4*	*5*	*6*
Virus and Broth	—	8/8	8/8	8/8	2/8	0/8
Virus and Duck Hepatitis Immune Serum	4/8	2/8	0/8	0/8	0/8	—
Virus and normal human serum (Infant)	—	8/8	8/8	6/8	1/8	—
Virus and Human Infectious Hepatitis Immune Serum	—	8/8	8/8	6/8	0/8	—

Numerator = number embryos with lesions
Denominator = number embryos infected

PROCEDURE

Our early studies of naturally infected ducklings were complicated by two factors. One was the presence of a second disease in some of the outbreaks (either paratyphoid or anatapestifer infection) and the other the fact that most of the tissues were collected from ducklings already dead of the disease. In view of the fact that one of the principal histological changes was necrosis of the liver parenchyma, we hesitated to evaluate these changes in dead birds. Accordingly, a controlled experiment was set up for the study of the pathogenesis of the disease.

Two groups of fifty each of three-day-old ducklings from a non-infected source were infected with chicken-embryo propagated virus (pool of 4th and 5th passages of MOD and OGE strains)—one group designated 1A, by intramuscular inoculation, the second group designated 1B, orally. A third group of birds designated 1C, was kept in a separate house as non-infected controls.

The entire experiment was repeated exactly as described, except that the ducklings in the second trial were seven days of age when they were infected. The ducks in experiment two were divided into three similar groups designated 2A, 2B, and 2C respectively. These ducks were from the same source and hatch as in experiment one and were infected with virus from the same frozen pool.

The infected ducklings were kept under almost continual observation. Visibly sick ducklings were removed from each group and sacrificed by decapitation for examination at periodic intervals. When no sick birds were available, they were selected at random. The intervals chosen were 8, 16, 24, 30, 36, 42, 48, 56, 64 and 72 hours after infection. All visibly sick ducks were examined when death seemed imminent. A group of the survivors was examined 8 days after infection. Control ducklings were also removed for examination at each of the selected time intervals.

For histological examination, liver, spleen and kidney and in a representative series also the brain, heart, lung and small intestine were collected from ducklings that had just been sac-

rificed. Thin slices were fixed in 10 percent aqueous formalin and in Bouin's solution. Blocks were embedded in paraffin and stained with hematoxylin and eosin. Selected sections were also stained with phosphotungstic acid-hematoxylin. For the demonstration of glycogen, paraffin sections were stained by the periodic acid-Schiff (PAS) technique, and compared with duplicate sections subjected to saliva digestion before they were similarly stained. Frozen sections were stained for lipid with Sudan blue black after fixation of the tissues in 10 percent aqueous formalin.

RESULTS

The symptoms, as well as the gross and microscopic lesions found in all of the infected ducklings in these two experiments were essentially similar. The most consistent and uniform results were secured with the ducklings in the first experiment inoculated intramuscularly (group 1A) and their corresponding control (group 1C). Fewer ducks developed symptoms and lesions in group 1B which was fed virus. Furthermore, the onset of the disease was delayed and the course more protracted. Only fifteen birds in group 1B became visibly sick while twenty from the comparable injected group (1A) developed symptoms. Similarly, the ducklings fed virus developed symptoms and gross lesions from 36 to 69 hours after exposure compared with 24 to 48 hours for the injected birds.

As was expected from the course of the disease in natural outbreaks, the effect of the virus inoculated or fed to seven-day-old ducklings (groups 2A and 2B) was less severe. Although fewer birds developed symptoms and gross lesions, the histological picture was essentially similar.

Since the results in the group (1A) of ducklings inoculated intramuscularly at three days of age were most consistent and typical of the disease pattern in all groups, this series was selected for the description of the symptoms and gross and microscopic pathology of the disease (table 3).

Symptoms. The symptoms observed in the inoculated ducklings were identical to those seen in the field outbreaks.

The birds first became dull and lagged behind the brood when driven around the pen. Their eyes assumed a slit-like appearance. They soon stopped moving and squatted down. Within a short time the ducklings fell on their sides, kicked spasmodically and assumed a position of opisthotonus. In field outbreaks and in previous experimental cases of the disease, deaths occurred within a half to one hour after symptoms were noted. In this experiment, the ducklings were sacrificed for examination as soon as they began to show signs of opisthotonus.

The first duckling became visibly sick 28 hours after infection. Almost all of the ducklings removed between 28 and 48 hours after infection were visibly sick. No sick ducklings were observed after 48 hours, and it is presumed that the birds sacrificed after this time would have survived the infection.

Gross pathological changes. The only consistent gross changes observed were in the liver. Although some congestion was noted in the spleen and kidneys this change was not consistent enough to be considered significant.

Normal duckling livers were reddish brown in color. Livers from affected ducks became enlarged up to twice normal size, very soft, and paler in color. In typical cases, the livers became almost yellow in color and were sprinkled with numerous petechial and ecchymotic hemorrhages. This gross lesion in conjunction with sudden death in ducklings up to 3 or 4 weeks of age, is essentially pathognomonic of this disease.

In the experimentally inoculated ducks, these hemorrhagic lesions were first observed 24 hours after infection in a duckling without clinical signs and selected at random. Practically all the ducks with symptoms or those sacrificed between 24 and 48 hours had typical liver lesions. The ducks removed and sacrificed at 56, 64, and 72 hours were clinically normal and had no gross liver lesions.

Two of five ducklings examined 8 days after infection had gross liver lesions. These lesions were not like those seen in the acute stage of the disease but consisted of an

irregular pale mottling of an otherwise normal appearing liver.

Histopathology. Examination of an extensive series of sections revealed no significant lesions in any organ examined except the liver. Therefore the following description is based on the series of liver slides examined.

Zero hours after inoculation—ducks 66, 68, and 70. There were no lesions in the control ducks examined at start of the experiment. However, there were two histological features observed in these livers that would be misleading to workers unfamiliar with the normal appearance of these organs. (see fig. 1, 2). First, the parenchymal cells in all parts of the lobules contained numerous vacuoles in the cytoplasm. These vacuoles varied in size, the average size being about two-thirds that of the cell nuclei. There were a few larger vacuoles suggestive of small fatty cysts, two or three times the diameter of the hepatic nuclei. Second, there were occasional mitoses of hepatic cells, which was not surprising in view of the rapid growth and development of ducklings at this age.

Eight hours after inoculation—control ducks 72, 74, 76, inoculated ducks 84, 86, 88. There were no recognized symptoms and gross or microscopic lesions in the control ducklings at this time or at any other time throughout the experiment. There were no recognized lesions in two of the three inoculated ducklings. The third inoculated duckling (86) had scattered heterophil leukocytes in the sinusoids.

Sixteen hours after inoculation—ducks 96, 98, 100. All three of the inoculated ducklings had recognizable lesions at this time. There were scattered heterophils present, several per hepatic lobule. These heterophils were not arranged in foci. Areas in the central zone of a few lobules were disrupted, contained fewer nuclei than normal and had indistinct cellular boundaries. These areas contained debris and some erythrocytes. Apparently these areas had softened and some sinusoids had ruptured allowing a small amount of hemorrhage.

Twenty-four hours after inoculation—ducks 114, 116. At this time one (116) of the two ducklings examined had lesions like those described at 16 hours. The other duckling,

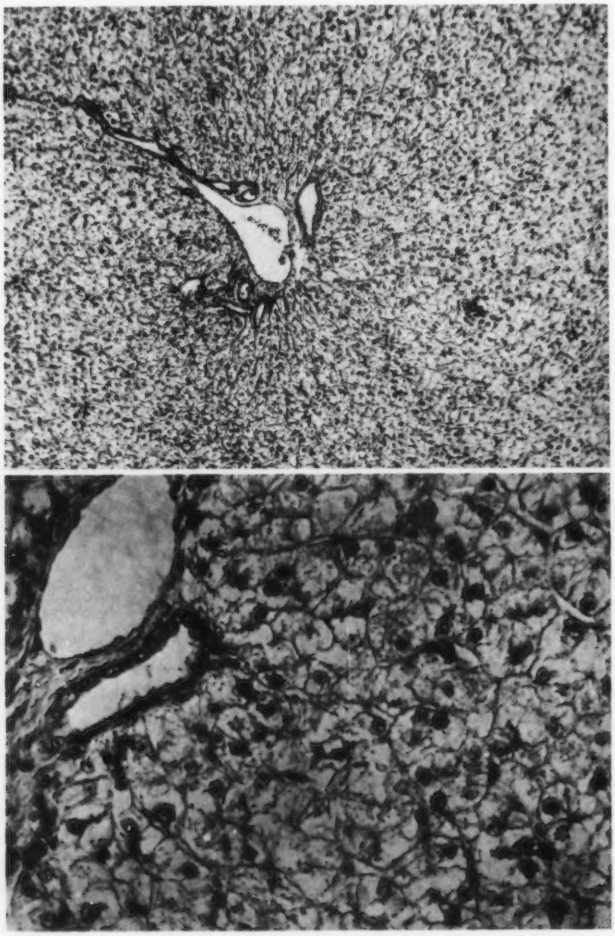

Fig. 1. (top) Section of liver from a normal three-day-old uninoculated duckling. Cytoplasm of parenchymal cells appears clear and vacuolated because of the high content of lipid and glycogen. Hematoxylin and eosin, 100×.

Fig. 2. (bottom) High power view of same section depicted in figure 1. Hematoxylin and eosin, 450×.

(114) although apparently clinically normal when selected for examination was the first one to have detectable gross liver lesions. This duckling also presented marked microscopic changes. There were very numerous heterophils, and in some places several were arranged along a sinusoid. Many of the hepatic parenchymal cells appeared necrotic. From 70 to 90 percent of the cells were necrotic in affected areas in different slides from the same duck. The cells immediately adjacent to the portal triads fared somewhat better than most, their nuclei frequently being morphologically intact. Other intact nuclei in cells with some basophilic cytoplasm were found scattered in the lobule, even near central veins. Many liver cords appeared completely necrotic and fragmented for considerable lengths. These were found both in the central and peripheral parts of the lobule. The necrosis did not have a clearly zonal distribution, but rather seemed to involve all of the parenchyma except for a few scattered cells in all zones. In places, completely necrotic and fragmented cords extended all the way to a central vein or portal canal, whereas scattered parenchymal nuclei in adjacent parallel cords were morphologically inact. The elongated, somewhat darker staining endothelial and Kupfer cell nuclei were apparently not involved. There were a few petechial hemorrhages. These occurred in areas where most of the hepatic cells were dead and the liver cords were somewhat disrupted. The blood appeared to fill in spaces where hepatic cells had disappeared. Some hepatic cells were interspersed between the erythrocytes. The hemorrhages did not distort the architecture of the lobules and were not related in their location to either the efferent veins or portal canals.

Twenty-eight and a half hours to 33 hours after inoculation—ducks 122, 124, 126, 134, 136, 138, 144, 146. These eight ducklings were recognizably sick when sacrificed and had characteristic gross liver lesions.

Microscopically the liver lesions were similar to those described in duck 114 except for some differences in degree (fig. 3, 4). Most of the hepatic cells showed evidence of necrosis. The bile duct and endothelial cells were not notice-

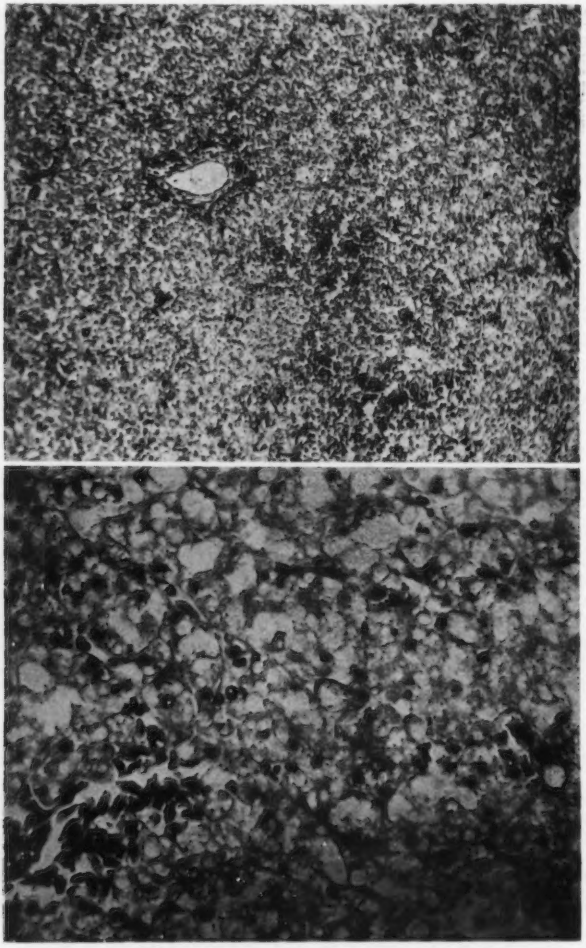

Fig. 3. (top) Section of liver of a duckling 30 hours after inoculation with duck hepatitis virus. Essentially all of the parenchymal cells are necrotic. The two darker areas in and below the center of the figure represent hemorrhages. Hematoxylin and eosin, 100×.

Fig. 4. (bottom) High power view of same section depicted in figure 3. Clear spaces represent areas of cell dissolution. Nuclear pyknosis and karolysis are evident in the parenchymal cells. There are some intact endothelial cells. There is hemorrhage and some disruption of architecture in the lower left hand corner. Hematoxylin and eosin, 450×.

ably changed. The greatest variation was in the extent of the hemorrhages. In some of the sections the hemorrhages were much larger, frequently involving confluent areas in several lobules. In such areas, the liver cord architecture was more severely disrupted.

Thirty-three and a half to 40 hours after inoculation—ducks 148, 150, 152, 170, 176. These five ducklings also were all recognizably sick when sacrificed and had characteristic gross liver lesions.

Miscroscopically the same liver lesions were found in this group as in the preceding group. These consisted of extensive necrosis of the hepatic cells and varying degrees of hemorrhage. In addition to these parenchymal lesions there were signs of an inflammatory reaction in the portal triads. These areas were edematous and contained numerous heterophilitic leukocytes and occasional foci of mononuclear cells. This inflammatory reaction was accompanied by bile duct proliferation involving almost every portal triad.

Forty-two hours after inoculation—ducks 178, 186. One (186) of these two ducks was not visibly sick and had no gross lesions when sacrificed. This duck had no apparent microscopic lesions. The other duck (178) was sick and had characteristic gross liver lesions. The microscopic lesions in this latter duck were similar to those described in the 33½ to 40 hour group.

Forty-eight hours after inoculation—ducks 206, 208. The first (206) of these two ducklings was slightly sluggish and had only a few small hemorrhagic spots on the liver when sacrificed. Microscopic examination revealed small areas of necrosis interspersed with larger areas where the hepatic cells were alive. The latter had normal looking nuclei and vacuolated cytoplasm but no hyperchromatism of the cytoplasm or mitotic figures. It gave the impression of a liver less damaged than the earlier specimens. Apparently some of the parenchymal areas were never necrotic. There was no evidence of an inflammatory response to indicate a clean-up of cellular debris. Bile duct proliferation was extensive and accompanied by numerous mitoses in the bile duct cells. The

general impression given by this slide was that this duckling survived because the liver parenchyma was less extensively damaged than in the preceding groups.

The second duckling (208) was free of symptoms and had only slight softening of the liver when sacrificed. The sections contained areas of dead parenchyma interspersed with areas of live cells. These liver cells were somewhat large and had hyperchromatic cytoplasm suggesting immaturity. These immature cells tended to extend along sinusoids (in one plane, on both sides of the sinusoid) for short distances, anywhere within the lobule. There was active bile duct proliferation accompanied by numerous mitotic figures. This was the first duckling in the series to reveal any evidence of liver cell regeneration.

Fifty-six hours after inoculation—ducks 218, 220. These two ducks had no symptoms and no gross lesions when sacrificed. The first of these ducks (218) presented a microscopic picture like that of duck 208 in the 48 hour group. There was regeneration of the parenchyma along the sinusoids and bile duct proliferation.

The second duck (220) presented a more complicated microscopic picture. In this bird, the liver cells were hyperchromatic, apparently young, with good cord formation, and with few vacuoles. These cells contained numerous mitotic figures, about two in each oil immersion field, in all parts of the liver lobule. There was extensive bile duct proliferation with many mitotic figures. In addition, there was active fibroblastic proliferation in the portal canals around the bile ducts. At the periphery of the zone of proliferating bile ducts, there appeared to be transitional forms between bile duct and parenchymal cells. The appearance suggested the formation of parenchymal cells from bile duct epithelium, a possibility that could not be discounted entirely in the liver of a bird five days old. On the other hand there was clear evidence that active regeneration was occurring from other parts of the lobule. There were small areas of necrosis in the midzonal position in the lobule, with active regeneration on the central side as well as the portal side of the area of necrosis. Furthermore, young

parenchymal cells surrounding some of the portal canals had no apparent connection with bile duct epithelium. This duck, apparently, must have recovered from extensive liver necrosis by regeneration of the liver parenchyma. Regeneration was essentially complete, as there were only a few small areas of necrosis left. In these areas, PAS stain suggested that the reticulum had been disrupted, whereas the reticulum and liver cord structure elsewhere in the liver appeared intact.

Sixty-four hours after inoculation—232, 234. There were no symptoms and no gross lesions in these two ducks. The only evidence of infection in the sections was a few, very small areas of necrosis. Such foci involved only about twelve to twenty cells in the plane of section, and were so infrequent that a careful search was necessary to find them. There was no bile duct proliferation. These ducks, apparently, had undergone only a very mild infection.

Seventy-two hours after inoculation—ducks 246, 248, 250. None of these three ducklings had symptoms and only one of the three (250) had slight softening of the liver. Sections from this latter duckling contained several areas of necrosis, a few large enough to involve a fifth of a lobule. There was hemorrhage in the necrotic tissue and regeneration at its periphery. Most of the parenchyma looked normal and there was only a small amount of bile duct proliferation.

The other two ducklings (246, 248) were essentially normal and had either developed a very mild infection or had not been infected at all. There was no evidence of necrosis, regeneration or bile duct proliferation in these sections.

Eight days after inoculation—ducks 438, 440, 442, 444, 446. None of the five ducks had symptoms and only two of the five had gross changes when sacrificed. The livers of the latter two ducks were slightly mottled in appearance. However, there seemed to be little correlation between these gross lesions and the microscopic findings since all five of the livers presented obvious lesions on histological examination.

The most striking change in the livers at this time was the bile duct proliferation (fig. 5, 6). The degree of prolifera-

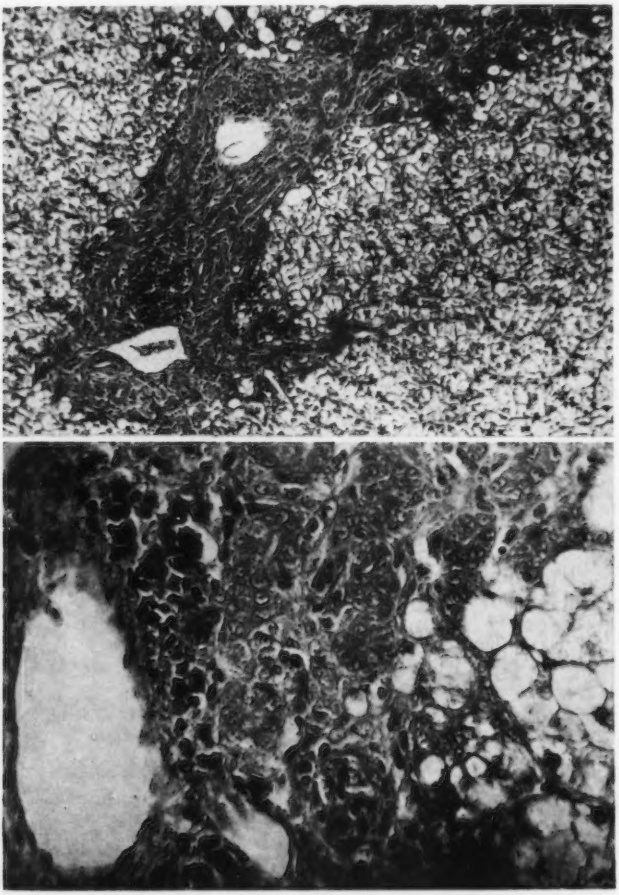

Fig. 5. (top) Section of liver of a duckling eight days after inoculation with duck hepatitis virus. Marked bile duct proliferation and focal accumulations of mononuclear cells in portal areas. Parenchymal cells appear normal. Hematoxylin and eosin, 100×.

Fig. 6. (bottom) High power view of same section depicted in figure 5. The proliferating bile duct cells in the center of the field have vesicular nuclei and abundant cytoplasm. The clump of smaller, darker cells between the bile ducts and the blood vessel represents an accumulation of mononuclear leucocytes. Note the fat and glycogen vacuoles in the parenchymal cells on the left. Hematoxylin and eosin, 450×.

tion was slight in one bird but well marked in the other four. Numerous mitoses were present in the mass of proliferating bile ducts. In a few slides that had marked bile duct proliferation, a few strands of cells resembling bile duct epithelium were found in the midzone or even near a central vein.

In two of the five ducks there were areas of focal necrosis. They were numerous in the bird that had only slight bile duct proliferation, suggesting that the process was not as far advanced in that bird. The necrotic foci varied in size from the area covered by several cells to areas somewhat larger than a liver lobule. Most were a fifth to a half of a lobule in area. There was no particular zonal distribution. The liver cells were completely necrotic. Hemorrhage had occurred into some of the necrotic areas. A few heterophils were usually found at the periphery of the necrotic tissue. Some necrotic areas were surrounded by parenchymal cells that were large, hyperchromatic and apparently regenerating (fig. 7, 8).

In some areas on most slides there were foci of inflammatory cells. These included a few heterophils but were composed mostly of mononuclear types. The latter were darker, smaller, and had less cytoplasm than the bile duct cells. These inflammatory foci occurred mainly in the portal areas. In one slide there were a few inflammatory foci in midzone or near a central vein.

Reexamination of sections collected from seven different natural outbreaks of the disease confirmed the impression that this experimentally produced disease was identical histologically to the disease as it occurred under field conditions.

A series of experiments were conducted to determine the nature of the numerous vacuoles present in the control ducklings at this age period. Liver sections from normal ducklings were examined after being stained for lipid and for glycogen. It was apparent that the livers of normal three-day-old ducklings contained a great deal of fat, including many large droplets. At five days of age, there was less fat and the droplets tended to be smaller. By the eleventh day of age the fat was much less prominent and was present as small droplets. There

TABLE III

Summary of experimental design and results in experiment #1 (groups 1A and 1C) wherein three-day-old ducklings were inoculated with duck virus hepatitis.

Hours after inoculation	Group 1C* Controls Duck numbers	Group 1A injected Duck number	Symptoms	Typical gross lesions of liver**	Microscopic lesions of liver
0	66, 68, 70				
8	72, 74, 76	84, 86, 88	—	—	scattered heterophils (1), negative (2)
16	102, 104, 100	96, 98, 100	—	—	heterophils, small areas of parenchymal necrosis (3)
24	108, 110, 112	114, 116	— —	—(1) +(1)	Heterophils, small areas of necrosis (1) heterophils, extensive necrosis and hemorrhage (1)
28½		122, 124, 126	+	+	heterophils, extensive necrosis and hemorrhage
30	128, 130, 132	134, 136, 138	+	+	heterophils, extensive necrosis and hemorrhage
32		144	+	+	"
33		146	+	—	"
33½		148	+	+	heterophils, extensive necrosis, hemorrhage, portal edema and bile duct proliferation
36	158, 160, 162	150, 152	+	+	"
39		170	+	+	"

40		176	+	+	"
42		178, 186	+(1) —(1)	+(1) —(1)	"(1) negative (1)
45		N.D.	+(1)	+(1)	N.D.
46		N.D.	+(1)	+(1)	N.D.
47		N.D.	+(1)	+(1)	N.D.
48	196, 198, 200	206, 208	+(1) —(1)	+(1) ±(1)	small areas of necrosis, bile duct proliferation (1) necrosis, parenchymal regeneration, bile duct proliferation (1)
56	212, 214, 216	218, 220	—	—	necrosis, regeneration, bile duct proliferation (1) regeneration, bile duct proliferation(1)
64	226, 228, 230	232, 234	—	—	few small areas of necrosis
72	240, 242, 244	246, 248, 250	—	—(2) soft(1)	negative(2) few small areas of necrosis, bile duct proliferation(1)
8 days	416, 418, 420 422, 424	438, 440, 442 444, 446	—	—(3) mottled (2)	few small areas of necrosis (2), mononuclear inflammatory foci (2) bile duct proliferation (3)

* Since no symptoms and gross or microscopic lesions were seen in group 1C, only the numbers of the birds examined is presented in this table.
** Typical gross lesions of liver. Pale, enlarged, soft and sprinkled with hemorrhages.
N.D. = not done

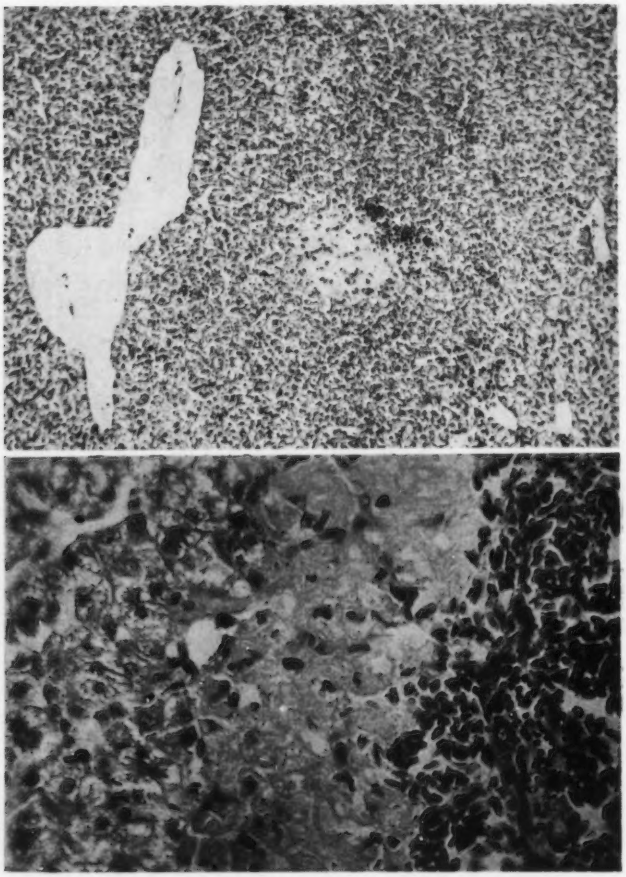

Fig. 7. (top) Section of liver of a duckling eight days after inoculation with duck hepatitis virus. Note the necrotic area in the center, which contains few nuclei. A small area of hemorrhage is evident at the right margin of the necrotic area. Hematoxylin and eosin, 100×.

Fig. 8. (bottom) High power view of same section depicted in figure 7. A normal zone of liver parenchyma is on the left of the figure. The central zone is composed of necrotic cells containing some pyknotic and broken nuclei. An extensive hemorrhagic area is seen on the right. Hematoxylin and eosin, 450×.

was a small amount of glycogen present as fine granules in the livers of three-day-old ducks, more at five days of age, and a great deal more at 11 days of age. Therefore, the vacuoles seen in hematoxylin and eosin stained paraffin sections of the livers of young ducklings represented both fat and glycogen. At three days of age, the vacuoles were largely fat, but with increasing age there was progressively less fat and more glycogen. A similar series of stains applied to liver sections of the ducklings inoculated with virus at three days of age revealed small droplets of glycogen in the control ducks and those examined eight hours after inoculation. The ducks inoculated 16 and 24 hours previously had scattered areas of liver deficient in glycogen. Glycogen was essentially absent in ducks that had been inoculated 28 to 40 hours previously, at the times when there was extensive parenchymal necrosis.

DISCUSSION

There are several remarkable features of this disease. One was the development of almost complete necrosis of liver parenchymal cells within 24 hours after inoculation. Another was the well marked bile duct proliferation within 33½ hours after inoculation. These indicated a very rapid evolution of the lesion and correspond with the occurrence of symptoms and gross lesions during the period of 24 to 48 hours after inoculation.

The intralobular distribution of the necrosis was difficult to determine because the lesion was so extensive that it usually involved the entire lobule. However, in none of the livers was there a zonal distribution of the necrosis, such as the centrolobular distribution seen in the viral hepatitides of man and the dog. Furthermore, there was evidence that when regeneration occurred from surviving cells, these cells could be situated in any zone of the lobule. This suggests that the intralobular circulation in the liver of the duckling may be somewhat different from that of the mammal. Perhaps some radicles of the hepatic artery may enter the sinusoids even farther from the portal area toward the central vein, than is the case in mammals.

Although most of the regeneration appeared to originate from surviving parenchymal cells, it was interesting that in a few instances, proliferating bile ducts appeared to go through a transitional form and then be continuous with regenerating parenchymal cells. The possibility that regeneration of parenchymal cells could take place from bile ducts in a five-day-old duckling was suggested.

ACKNOWLEDGMENTS

We wish to express our deep appreciation to Dr. Peter Olafson for advice and help in many aspects of this problem. Thanks are also due to Dr. A. V. Machado, Dr. E. Dougherty III and the Long Island Duck Growers Research Cooperative for assistance in phases of this work.

SUMMARY AND CONCLUSIONS

Virus neutralization tests with convalescent sera from human and canine virus hepatitis, as well as normal human and canine sera, failed to demonstrate any serological relationship of these diseases to duck virus hepatitis.

The pathology and pathogenesis of virus hepatitis of ducks was studied in two experiments with experimentally infected ducklings. The significant gross pathological changes were confined to the liver. This organ became enlarged, pale, soft, and sprinkled with petechial and ecchymotic hemorrhages.

Microscopically, the primary changes consisted of necrosis of the parenchymal cells of the liver, and proliferation of the bile duct epithelium. These changes were accompanied by varying degrees of inflammatory response and hemorrhage. In ducklings that did not develop symptoms or die, successful regeneration of the liver parenchyma was observed. There was a strong indication that regeneration of parenchymal cells proceeded not only from surviving parenchymal cells but also from bile duct epithelium.

These pathological changes in the experimental birds were essentially similar to those observed previously in natural infections.

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ORAL THERAPY FOR ERYSIPELAS IN TURKEYS*

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FOR THE past several years some workers have been recommending the use of penicillin in the therapy of swine erysipelas. Some[11] suggest giving it to affected swine simultaneously with anti-serum since relapses are reported follow-

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ing therapy with penicillin alone. According to Muller,[11] experience and research in Germany justify the conclusion that the use of penicillin is "absolutely" imperative. Aitken[1] in a recent editorial commented that Muller's report endorses American findings on the remarkable therapeutic value of penicillin in acute swine erysipelas.

Gifford and Jungherr[5], Grey[6], and Prier and Alberts[13] have shown that penicillin is effective against both experimental and naturally occurring erysipelas in turkeys. The recommended treatment for this disease in turkeys at present is to give affected birds 100,000 units or more of penicillin intramuscularly. Experience has shown this treatment to bring about remarkable recoveries in the majority of sick turkeys. It does not, however, prevent additional birds from becoming sick and is of value only in those birds with symptoms. If oral therapy were successful it would be of considerable value in preventing the infection from spreading in affected turkey flocks and in bringing outbreaks under control.

Ullberg[16] mentioned that penicillin, aureomycin and terramycin might prove of value in erysipelas therapy if put into the feed, but that there was little reason to assume that aureomycin or terramycin would prove to be more effective than penicillin. Since then, several workers[8,9] have reported on trials with oral therapy in erysipelas of swine and turkeys. Moynihan and Stovell[10] reported promising results with oral antibiotic therapy on small numbers of turkeys. Schneider, Spencer and Ensminger[14] on experiments with swine reported that penicillin or oxytetracycline fed at the rate of 15 mg per pound of feed had no effect on the symptoms or lesions of erysipelas infected pigs. They also fed penicillin in a ration to pigs at a level of 40 mg per pound of feed and it failed to inhibit the symptoms or lesions of erysipelas, as measured by skin changes and temperature levels. Orlandella[12] found that erythromycin given subcutaneously or per os was effective in treating experimental erysipelas in pigeons. H. W. Smith[15] in experiments with oral therapy in chicks concluded that chloramphenicol, terramycin, aureomycin and furazolidone

were of little value in experimental erysipelas infections. Elliott[4], in a natural outbreak in caged turkeys, tried oral penicillin therapy on an uncontrolled basis. He fed all the birds (100) feed containing 250 gm/ton procaine penicillin for one week, 150 gm/ton for a second week and 80 gm/ton for a third and final week. Three turkeys died in a four day period prior to this medication and one died after the penicillin feed was started. An editorial comment on this case mentions that it might be misleading to credit the feeding of penicillin for the disappearance of the erysipelas infection for two reasons: (1) sudden appearance and then abatement of acute erysipelas is a common occurrence, at least in swine, and (2) no benefit has resulted from feeding penicillin to pigs when acute erysipelas has been induced experimentally. Jerstad and Johns[7] reported that furazolidone at the 0.02 percent level in feed had slight, if any, effect on experimental erysipelas of turkeys. Boyer and Brown[2] obtained some very promising results with procaine penicillin in the feed against experimental erysipelas in turkeys. Their results with aureomycin were less encouraging from the standpoint of morbidity.

It is the purpose of this paper to present additional experimental evidence on the effect of oral antibiotic therapy against experimental erysipelas in turkeys, and to report on the results obtained in three controlled field trials with natural outbreaks.

MATERIALS AND METHODS

The strain of *E. rhusiopathiae* used in all of these experiments was the same as reported previously.[2] The number of dead birds necropsied and cultured and methods used for isolating *E. rhusiopathiae* as well as the criteria used for its identification were the same as reported previously.[2,3]

All infected birds in these trials were inoculated with 0.1 ml intramuscularly (breast) of a 24-hour chicken infusion broth culture that had been seeded with experimental infected turkey livers. Medications were incorporated into the all mash turkey ration with an electric mixer. All medications were started at the time of inoculation with the infecting organism.

were continued for 10 days post-inoculation and then discontinued. Observations of the experimental birds were continued for an additional two weeks after medication was stopped. Observations on the number of sick birds were recorded twice daily, morning and late afternoon. Daily records of the consumption of all feed, water, or both were kept. The antibiotic intake (mg per lb body weight) was then calculated on the basis of the average weight of the birds in each experimental group.

Daily temperatures and general weather observations were recorded. Insofar as was possible rain was prevented from getting into feed and water containers on the ranges or open porches. If birds had access to feed or water other than that offered, this was noted.

Experiments with young poults were done inside buildings in either starting or holding type batteries. The experiments with adult turkeys were conducted in houses with small porches; on large open porches with wire floors, or in large outside yards 45 by 50 feet with gravel on the ground.

RESULTS

In a previous publication[2] we mentioned that the drug intake calculated as mgs per lb. of body weight was much greater (three to five times) in infected young poults than in adult birds. We repeated this work and again found this to be true when poults 7 weeks of age were compared with 20-week-old turkeys. This held true with parallel pens of non-medicated, non-infected controls and with parallel pens of birds given procaine penicillin in the feed at 600 gm/ton and in the water at 1200 mgs/gal. The young poults' drug intake in mg/lb. body weight was approximately four times greater than in the older birds. Table 1 is a summary of these results.

Three preliminary pilot trials using 392 poults 4 to 8 weeks of age medicated with the following antibiotics or combinations in the feed, in the water or both, gave *inadequate* protection:

1. Aureomycin 100 gms/ton plus procaine penicillin 400 mgs/gal

2. Aureomycin 100 gms/ton plus furazolidone 150 gms/ton plus procaine penicillin 400 mgs/gal
3. Aureomycin 100 gms/ton plus terramycin 100 gms/ton plus procaine penicillin 400 mgs/gal
4. Aureomycin 200 gms/ton
5. Aureomycin 100 gms/ton plus terramycin 100 gms/ton
6. Aureomycin 100 gms/ton
7. Bacitracin 200 gms/ton plus procaine penicillin 40 gms/ton plus procaine penicillin 240 mgs/gal
8. Bacitracin 400 gms/ton plus procaine penicillin 80 gms/ton
9. Aureomycin 100 gms/ton plus furazolidone 100 gms/ton

The following antibiotics or combinations gave the best results in pilot trials on young poults thus justifying further investigation with older birds:

1. Procaine penicillin 1200 mgs/gal
2. Procaine penicillin 300 to 600 gms/ton
3. Aureomycin 325 gms/ton
4. Aureomycin 200 gms/ton plus procaine penicillin 800 mgs/gal
5. Aureomycin 200 gms/ton plus aureomycin soluble 800 mgs/gal
6. Aureomycin 200 gms/ton plus procaine penicillin 400 mgs/gal

Six controlled laboratory experiments using 505 turkeys 20 to 48 weeks of age were done. The results of two of these trials were invalidated for two reasons. In one experiment mortality in the non-medicated, infected controls was only 43 per cent which we did not consider a severe enough exposure to evaluate the antibiotics satisfactorily. In the other trial conducted on open porches, the medicated feed and water became thoroughly soaked and diluted from 5 days of heavy rains. The other four controlled laboratory experiments were done during the months of July, November, December and January respectively.

Figure 1 shows the antibiotic intake in mgs/lb of body weight and its relation to morbidity and mortality from ex-

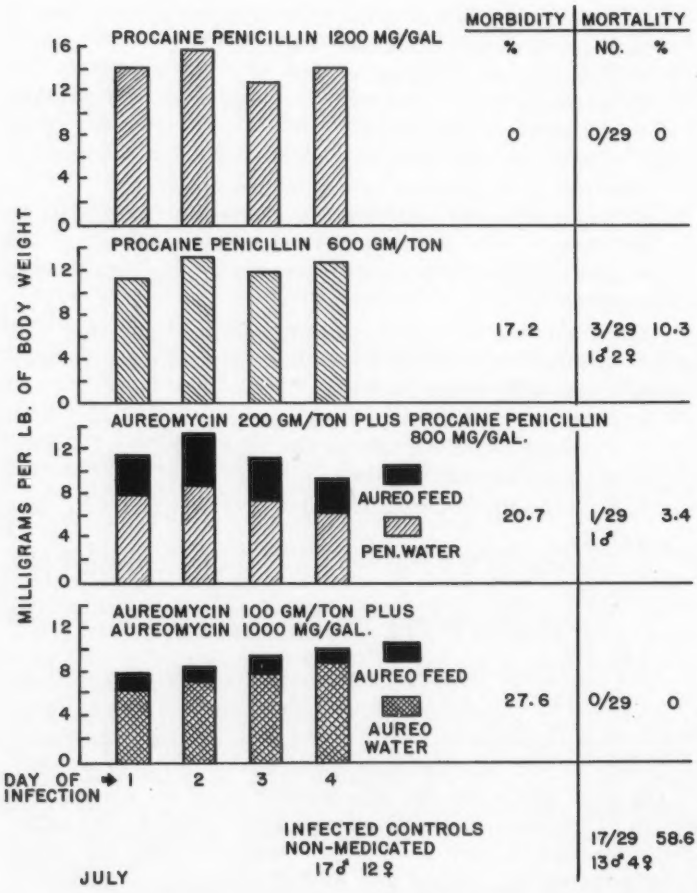

Fig. 1. Milligrams of antibiotic intake per pound of body weight in relation to morbidity and mortality during summer weather for experimental erysipelas in turkevs 20½ weeks of age.

perimental erysipelas in 20½-week-old turkeys during summer weather. The antibiotic intake for the first four days is indicative of the trend of drug intake for the rest of the medication period. Most of the mortality occurred during this same four day period in all experiments. Good results were obtained in this experiment with all of the treatments when compared with the infected, non-medicated controls. The best results concerning both morbidity and mortality and with the highest antibiotic intake were obtained with 1200 mgs/gal procaine penicillin. From the standpoint of mortality, only the pen on aureomycin at 100 gm/ton plus 1000 mgs/gal also gave 100 per cent protection. However, this pen had the highest morbidity and the lowest total antibiotic intake. The total antibiotic intake was more nearly alike with the other two treatments. The morbidity and mortality figures also were quite close.

The morbidity and mortality resulting from two levels of procaine penicillin in the drinking water during winter weather with experimentally infected turkeys 21 and 20 weeks of age respectively is shown in table 2. Despite the fact that more penicillin was offered (3.0 gm/gal) and the challenge not as great, (62.5 percent mortality in the non-medicated controls compared to 82.6 percent in the controls on 2 gm/gal) more birds got sick (47 percent) and more died (29.4 percent) with the 3 gms/gal level.

In table 3 we see the results with a still higher (4 gm/gal) level of procaine penicilin given in the water. The first treatment group was supplied the medicated water *ad lib*. However, in two of the treated groups the drinking water medication was only available to the birds for 4 hours out of each 24, from 8:00 to 10:00 A.M. and from 2:00 to 4:00 P.M. This restricted water intake was an attempt to stimulate farm conditions in the winter when water freezes and becomes available to the turkeys only when the poultryman replaces it. One of these groups on restricted water intake was supplemented with 500 gm/ton in the feed *ad lib*. In this experiment, the highest antibiotic intake was in the group given procaine penicillin at 500 gm/ton *ad lib* plus 4 gm/gal for 4

TABLE I

Antibiotic intake per pound of body weight when given in the feed and water in September to turkeys of different ages experimentally infected with *Erysipelothrix rhusiopathiae*.

Day of infection	Procaine penicillin 600 gm/ton (mg per lb body weight) 20 wks. age	7 wks. age	Procaine penicillin 1200 mg/gal. (mg per lb body weight) 20 wks. age	7 wks. age
1	9.66	38.85	10.35	29.54
2	9.66	39.93	9.77	34.36
3	11.15	45.32	9.43	36.64
4	11.84	48.56	9.66	41.46
Total	42.31	172.66	39.21	142.00
Ave.	10.58	43.17	9.80	35.50

TABLE II

Effect of two levels of procaine peniccillin** in the drinking water during winter weather on morbidity and mortality of adult turkeys experimentally infected with *Erysipelothrix rhusiopathiae*.

TREATMENT	PENICILLIN INTAKE (mg per lb body weight) Day of infection* 1	2	3	4	MORBIDITY (peak day)	MORTALITY No.	%
2 gms/gal.	10.9	10.8	9.8	11.4	26.1%	2/23	8.7
Infected non-medicated controls 9M 14F					65.2%	19/23 9M 10F	82.6
*Weather: dry and moderately cold. (Nov.)							
3 gms/gal.	6.8	8.7	9.0	8.5	47.1%	5/17 2M 3F	29.4
Infected non-medicated controls 8M 8F					75.0%	10/16 6M 4F	62.5
* Weather: snow and cold. (Dec.) Snow available to birds first 6 days of trial.							

M = Male F = Female
** Penline—200; Commercial Solvents Corp.

hours per day. These turkeys had the lowest morbidity but suffered one more death than the group getting 4 gm/gal *ad lib*. Oddly enough, this group had the highest morbidity (47.6 percent) but the lowest mortality. The pen receiving 4 gm/gal for 4 hours per day had the lowest antibiotic in-

TABLE III

Effect of procaine penicillin** intake on morbidity and mortality during winter weather in adult turkeys experimentally infected with *Erysipelothrix rhusiopathiae*.

TREATMENT	PENICILLIN INTAKE (mg per lb body weight) Day of infection* 1	2	3	4	MORBIDITY (peak day)	MORTALITY No.	%
4 gms/gal. (ad lib.)	13.1	6.8	18.0	17.5	47.6%	2/42 2F	4.8
4 gms/gal. (4 hrs. day)	10.1	8.6	15.1	17.6	33.3%	6/42 2M 4F	14.3
500 gms/ton plus 4 gms/gal. (4 hrs. day)	14.1	13.0	20.1	22.3	26.2%	3/42 2M 1F	7.1
Infected non-medicated controls					95.2%	35/42 13M 22F	83.3
14M 28F							

* Weather: snow and cold (Jan.)
Snow available to birds first and second days of trial.
M=Male F=Female
**Penline-200; Commercial Solvents Corp.

take, the next highest morbidity and the highest mortality. During this experiment the weather was cold and the birds ate the snow on the ground during the first 2 days of the trial.

Table 4 gives a summary of the best results obtained with adult turkeys during the summer and winter experiments. The antibiotic intake from water per lb body weight during the summer was much greater than during the winter even though more of the antibiotic was in the water. When the medication is in the feed, the intake is less during the hot weather.

The results of the controlled field trial in table 5 are divided into three parts on the basis of medication and environment. This trial involved 1300 turkeys which had suffered heavy mortality for 2 weeks. However, birds were not submitted for laboratory examination until several hundred had died.

TABLE IV

Summary of best results with oral therapy in experimental erysipelas of adult turkeys during summer and winter.

Treatment	Daily intake in mg per lb. of body weight	Mortality dead/total	%	Mortality Non-med. controls dead/total	%
Summer Experiments (July).					
1. Procaine penicillin* 1200 mgs/gal.	15.52	0/29	0	17/29	59
2. Aureomycin** 100 gm/ton plus Aureomycin soluble† 1000 mgs/gal.	10.06	0/29	0	17/29	59
3. Aureomycin** 200 gm/ton plus procaine penicillin* 800 mgs/gal.	13.32	1/29	3.4	17/29	59
4. Procaine penicillin* 600 gms/ton	12.96	3/29	10.3	17/29	59
Winter Experiments					
1. Procaine penicillin* 2.0 mg/gal. (Nov.)	11.36	2/23	8.7	19.23	83
2. Procaine penicillin* 800 gm/ton (Dec.)	30.41	0/10	0	8/10	80
3. Procaine penicillin* 4.0 gm/gal (Jan.)	18.04	2/42	4.8	35/42	83
4. Procaine penicillin* 500 gm/ton ad lib plus Procaine penicillin* 4.0 gm/gal 4 hrs./day (Jan.)	22.30	3/42	7.2	35/42	83

*Penline-200; Commercial Solvents Corp.
†Aureomycin Soluble; American Cyanamid Co.
**Aureomycin T.F., American Cyanamid Co.

Our trial began two days after the diagnosis of erysipelas was made. The birds were on the ground floor of a two story barn. They were divided into a non-medicated control group of 300 (150 males and 150 females) and two procaine penicillin

TABLE V

The effect of 3.6 gm/gal. water of procaine penicillin* during severe winter weather on mortality in turkeys with natural erysipelas.

Treatment	Mortality No.	Mortality %
Non-medicated exposed controls 300 (150 M 150 F) moved to clean quarters	55/300 33M 22F	18.3
Medicated exposed turkeys 300 (mixed sex) moved to clean quarters	2/300 2M	0.66
Medicated turkeys 700 (mixed sex) kept in original contaminated quarters	11/700 4M 7F	1.57

M=Male F=Female
* Penline-200; Commercial Solvents Corp.

medicated (3.6 gm/gal) groups of 700 and 300 (mixed sex) respectively. The non-medicated control group of 300 was, of necessity, moved to a pen occupying one-half of the second floor. This was essentially a clean environment insofar as erysipelas was concerned. The larger medicated group of 700 remained in the original, contaminated pen on the ground floor with straw litter. The smaller lot of 300 was moved up to the second floor. The birds in each of the groups were divided proportionately on the basis of sex and symptoms. Results showed that 55 of the 300 non-medicated controls died (18.3 percent) compared to two from the smaller medicated group (0.66 percent) and 11 of the larger medicated group (1.3 percent). This trial was run in December and January during severe winter weather in Northern New York.

DISCUSSION

In two similar experiments, we have shown that the antibiotic intake calculated in mgs per lb of body weight is about four times greater in young poults than in adult turkeys. In our latest experiments this was confirmed in both non-infected controls and in infected, medicated birds. Young, growing poults have greater feed and water intakes per pound of weight than adults. Intake of medications administered

in feed or water will also be greater in younger turkeys. This explains why the mortality results in a number of our pilot trials were often better with poults than with the same drug levels in adults.

One observation we made consistently was that some aureomycin medicated groups had a low mortality despite a lower antibiotic intake and a higher morbidity than other medicated groups in the same experiment. Many of these sick birds remained visibly sick for a number of days or a week and then slowly recovered. Although the mortality in these aureomycin treated groups was low, we do not feel that aureomycin can be judged a better treatment than other antibiotics which induced a lower morbidity even though a slightly higher mortality may have occurred.

The weather certainly has considerable influence on feed and water intake in turkeys and possibly on medication given in the feed or water. One can see that the antibiotic intake in July with 1200 mgs/gal (figure 1) is greater than the intake in November with 2.0 gm/gal (table 2) in birds of comparable age. However, the July experiment consisted of 12 hens and 17 toms whereas the November experiment was made up of 14 hens and 9 toms. Since toms usually eat and drink more than hens of the same age, it could be that the intake difference is due to this factor as much as or more than the weather. Experiments are now in progress to settle this point. Since toms usually eat more than hens, they would take in more antibiotic but not necessarily on a mgs/lb of body weight basis. But assuming that they may take in more on a mgs/lb body weight basis, then they would receive, theoretically, more antibiotic protection. There were 49 adult toms in our four experiments of which eight died (16.3 percent). There were 62 hens of which 14 (22.5 percent) died. Although approximately 6.0 percent more medicated hens died than toms, this difference probably is not significant. The mortality in the non-medicated infected toms was 41 out of 48 (85.4 percent) and 40 out of 62 hens (64.5 percent), a difference of about 20 percent. In these experiments, our mortality in non-medicated infected males was slightly above the 80 percent we

reported previously.[2] The mortality in the hens reported here was slightly less than our previously[2] reported figure of 67 percent.

The results of the controlled field trial seem to show that moving the birds to a clean environment reduced the mortality slightly since only two died out of 300 in the medicated group moved upstairs to a clean pen, whereas 11 out of 700 died of those left downstairs in the original, contaminated pen. However, with figures so small, one cannot be sure that the environment made a significant difference. If it did, then it was in favor of the treatment, because all of the 300 nonmedicated controls had likewise been moved to similar clean quarters and thereby had their exposure reduced. Despite this possibility, 55 out of these 300 controls died, a mortality of approximately 12 times greater than in the large medicated group of 700 left in the contaminated pen and 28 times greater than in the comparable group of 300 medicated birds in a similar clean environment. It should also be stressed that this field trial was done under severe weather conditions even though the birds were under cover. The temperature during at least half of this experiment hovered between 10 above and 15 below zero. This severe cold certainly reduced the water intake considerably since the medicated water was only mixed up once a day and frequently it was frozen solid within several hours and discarded as ice the next day. It should also be mentioned that the form of procaine penicillin used was Penline–200.* This is made to be used in feed and contains some insoluble vehicle. Despite this fact, we used it in water in all of our laboratory experiments and in this field trial. One lot of a soluble procaine penicillin product provided was tried and apparently was not as palatable as Penline–200, based on results of a pilot trial. Our experimental results indicate that the active ingredient of Penline—200 which is procaine penicillin, was certainly soluble. Actually there was not very much of any insoluble sediment when mixed with water.

* Commercial Solvents Corporation.

From the standpoint of experimental results and cost, we feel that procaine penicillin is the most practical and efficient antibiotic to use for erysipelas control. The dosage whether in the feed or water will have to be computed on the probable intake. This will depend on weather, sex, and other conditions. The favorable result of oral medication in the field trial corroborates our results in the laboratory experiments.

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Thanks are due to American Cyanamid Company (Farm & Home Division) and to Commercial Solvents Corp. for supplying antibiotics and financial assistance in carrying out this study.

SUMMARY

1. The efficiency of antibiotics in feed and water for controlling erysipelas in turkeys was determined in eleven controlled laboratory experiments and three controlled field trials. In all 6162 turkeys ranging in age from 4 to 48 weeks were involved. In the laboratory experiments birds were challenged with 0.1 ml of a 24-hour infusion broth culture of *Erysipelothrix rhusiopathiae* intramuscularly. The field trials involved natural outbreaks. Various levels of aureomycin, terramycin, bacitracin, procaine penicillin, and furazolidone were tried singly, and in combination in feed, water or in both in pilot trials with young poults. Investigations with adult turkeys were limited to those antibiotics or combinations that gave the best results in young poults.

2. Experiments confirmed our previous findings that the drug intake calculated as mg per pound of body weight was approximately four times greater in young poults than in older birds.

3. Best results during summer weather were obtained with a) procaine penicillin in the water at 1200 mgs per gallon, b) procaine penicillin in the feed at 600 gms per ton, c) with aureomycin 100 gms per ton in feed plus aureomycin

soluble in the water at 1000 mgs per gallon, and d) with aureomycin at 200 gms per ton in feed plus procaine penicillin 800 mgs per gallon in the water. Best results during winter weather were obtained with e) procaine penicillin in the water at 2.0 and at 4.0 gms per gallon, f) procaine penicillin in the feed at 800 gms per ton, and g) procaine penicillin at 500 gms per ton in the feed *ad lib* plus 4.0 gms per gallon in the water for 4 hours each day.

4. Three controlled field trials were conducted with natural outbreaks in turkeys 20 to 26 weeks of age. In two of the trials the mortality in the non-medicated controls was too low to evaluate the efficacy of the antibiotics satisfactorily. The third trial was done during severe winter weather and involved 1300 turkeys. The flock was divided into a non-medicated control group of 300 (150 males and 150 females) and two groups of 700 and 300 (mixed sex) each medicated with 3.6 gms procaine penicillin per gallon water. Results showed that 55 of the 300 non-medicated controls died (18.3 percent) as compared to 13 of the 1000 in the two medicated groups (1.3 percent). The favorable result of oral medication in this field trial corroborated our results in the laboratory experiments.

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SELECTED NEOPLASMS IN EXOTIC BIRDS*

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NEOPLASTIC diseases of birds represent a vital segment of veterinary pathology. Similarly, comparative oncology, embracing exotic species of captive birds, merits our attention. The main facts pertaining to tumor genesis are well known.[19,23] It is generally held that the malignant cell develops from the normal cell by mutation.[1] Practically any cell can be-

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come neoplastic if it can reproduce itself. Ewing[4] said: "Cancer is not one disease but a thousand different diseases." Future research may well support his statement.

It is obviously impossible to classify accurately neoplasms on an etiological basis. In fact, no perfect classification system exists at the present time.

Considerable variation exists relative to the incidence of neoplasia in the common domestic fowl. Tumor-incidence in birds is difficult to measure. Information concerning the age of the birds, length of period covered by a survey, and certain unknown factors unquestionably affect the results. Nevertheless, the normal death rate among chickens is high compared to other species. Consequently, large numbers of domestic poultry reach the necropsy table. Many of them are tumor-bearing individuals.

Neoplastic diseases are probably more common in the domestic hen than in any of the other domesticated species.[5] Schnieder[19] reported a two to three percent incidence of tumors in birds between 6 and 18 months old. However, no microstudies were made on these birds. Among 24,000 birds at necropsy, Goss[11] found 6 percent to be affected with neoplasms. In general, it may be assumed that chickens less than six months old are seldom victims of neoplasia. Between one and two years of age, the incidence of neoplastic diseases is often surprisingly high. An annual increase of two to three percent in birds one year old or less is approximately correct, with a marked increase in frequency in the second year of life.[17] Certain forms of avian connective tissue tumors are transmissible by filter-passing agents. An excellent review by Claude and Murphy[2] adequately covers this phenomenon. Unquestionably, neoplasia of the lymphoid cell system is the most common tumor entity in chickens.[8]

Tumors, both benign and malignant, are well known in both domestic and captive wild birds. Innocent neoplasms such as papillomata or lipomata are common and cause death by pressure, obstruction, or displacement of vital viscera.

The classical contributions of Fox[10] to diseases of wild mammals and birds are unequaled. He reported 37 neoplasms in captive birds, most of which involved the genito-urinary

system. Ratcliffe[16] found 82 tumors in 81 birds. The highest incidence was in the Psittaciformes with a total of 42 tumors. The Galliformes showed the second highest incidence. Most of the avian neoplasms in his study involved the genito-urinary organs and were of epithelial origin. Tissues of the digestive tube and related structures were also frequent sites of tumor genesis. Ratcliffe concluded that the incidence of neoplasia in captive mammals and birds depends upon (a) the average age of the group and (b) the resistance or susceptibility to neoplasia. As age increases, tumor incidence likewise increases. Pituitary tumors are exceedingly common in parrakeets.[18] The incidence of this endocrine tumor is apparently equal in both sexes. Most of the neoplasms are locally invasive and reduce the average life span of the affected parrakeet by six and one half years. In addition, 10 percent of the birds with pituitary tumors also had associated renal adenocarcinomata. O'Connor[15] has reported an epidermal carcinoma of the wing of a bald eagle, an adenocarcinoma of the thyroid in a shell parrâkeet, and an abdominal lymphosarcoma in a great blue heron. Wadsworth and Hill[21] described a theca-cell tumor of the ovary in a budgerigar as well as a fibrosarocoma of the thoracic wall in a ring-necked parrakeet. Recently, Halloran[12] has compiled a complete bibliography of references to diseases of wild mammals and birds. This review of the literature is a priceless contribution to veterinary medicine as it relates to captive species.

Although tumors do occur in comparatively young birds, the preponderance of cases are recorded in aged birds or at least in those having spent long years in captivity. The prevalence of neoplasia in the parrots, known to endure captive condition over long periods, bears out this fact. According to Hill,[13] species prevalence appears to be mainly associated with tolerance of captivity combined with known natural longevity. During the last 20 years, he has encountered 42 cases of avian tumors (excluding leucosis). Twenty of them involved psittacine birds, of which 9 were burgerigars. The next most frequently affected were the Passerines (6 cases), followed by plovers (5), pheasants (3), anserines and pelecaniforms (2 each), and finally single examples from each of the fol-

lowing orders: Accipitrines, Coraciformes, Pigeons, and Ratites. Lipomata were most frequently encountered. These tumors were usually subcutaneous growths, single or more commonly multiple, becoming pendulous and pedunculated, and causing discomfort. Consequently, the bird pecked the lesion, resulting in irritation and secondary sepsis. Death usually ensues from pressure effects, especially if the growths are multiple and involve the mesenteric fat.

The purpose of this paper is to describe and illustrate a few selected neoplasms from exotic birds sent to the senior author for diagnosis. Each specimen was submitted by Dr. W. C. Osman Hill of the Zoological Society of London, England.

Pennant's parrakeet *(Platycercus elegans)*

This male bird, weighing 95 grams, had been exhibited in the London Zoo menagerie for nearly 11 years. Although well nourished and in excellent plumage, he died of senility and secondary injuries. The right tibia was greatly swollen and covered with a hemorrhagic exudate, the latter congealed over an extensive area of the skin. Necropsy revealed the subcutaneous tissues to be healthy, except over the right tibial region, where there was a large, opaque, yellowish-white tumor partly embedded in the musculature. The liver was very pale and cirrhotic. Anemia was evident, secondary to chronic small hemorrhages. A bilocular, fluid-filled cyst was present between the liver and pericardium. Its contents were gelatinous. Microstudies revealed both the cyst wall and contents to be apparently structureless. The kidneys were very pale with some evidence of urinary retention. Microscopic examination of the tumor revealed nests of cells resembling squamous epithelium within a connective tissue stroma (fig. 1). Mitosis was evident. The diagnosis was epithelioma. Jackson[14] refers to this type of neoplasm as an acanthoma, and considers it among the most rapidly growing of all tumors. An epithelioma is a squamous cell carcinoma of the skin. These tumors differ widely in their malignancy and metastasis.

Leadbeater's cockatoo *(Lophochroa leadbeateri)*

This specimen weighed 365 grams and had been exhibited

at the London Zoo for 20 years. The cause of death was a malignant disease. External examination of the bird revealed it to be slightly cachectic. Its abdomen was protruberant and tense. Necropsy revealed some edema of the subcutaneous tissues with severe ascites of the peritoneal cavity. The digestive tube appeared normal, but contracted. Several coils of intestine were adherent to a large tumor mass in the mesentery. The liver was grossly enlarged and extensively replaced by opaque tumor tissue. A large tumor mass also occupied the site of the mesenteric lymph nodes. Microscopic studies revealed a malignant hepatoma (fig. 2). Two main types of carcinomas involve the liver, (a) liver-cell carcinoma or malignant hepatoma and (b) adenocarcinoma of the bile-duct type or cholangioma. The liver-cell carcinoma, as in this case, often displays a structure similar to normal liver tissue. However, the proper lobular pattern is lost. Anaplasia with great cellular pleomorphism is not uncommon. Intra-hepatic portal metastasis occurs frequently.[14] Invasion of hepatic veins inevitably produces pulmonary metastases. Hepatic carcinomata are not rare in cattle, sheep, and dogs.[6] This neoplasm is less common in cats, horses, pigs, and other domestic species. A few cases have been reported in domestic fowl.[9]

Cut-throat finch *(Amadina fasciata)*

This individual was exhibited in the London Gardens for five and one-half years. Its body was cachectic and in poor plumage. The right humerus revealed an irregularly-nodular, firm tumor extending from the edge of the pectoralis major insertion to the elbow, and projecting on the dorsal and ventral surfaces of the wing. On section, the tumor cut with difficulty, having nearly the same consistency as bone. The only visceral pathology involved the liver. This organ was very pale, rather hyaline in appearance, and presented a few, small, whitish lesions on the anterior dorsal surface. Microstudies of the bone lesion revealed it to be an osteoma (fig. 3). The majority of osteoblastomas arise from the bones of the head and extremities. Considerable controversy exists relative to the true nature of bone tumors. Inflammatory or degenerative

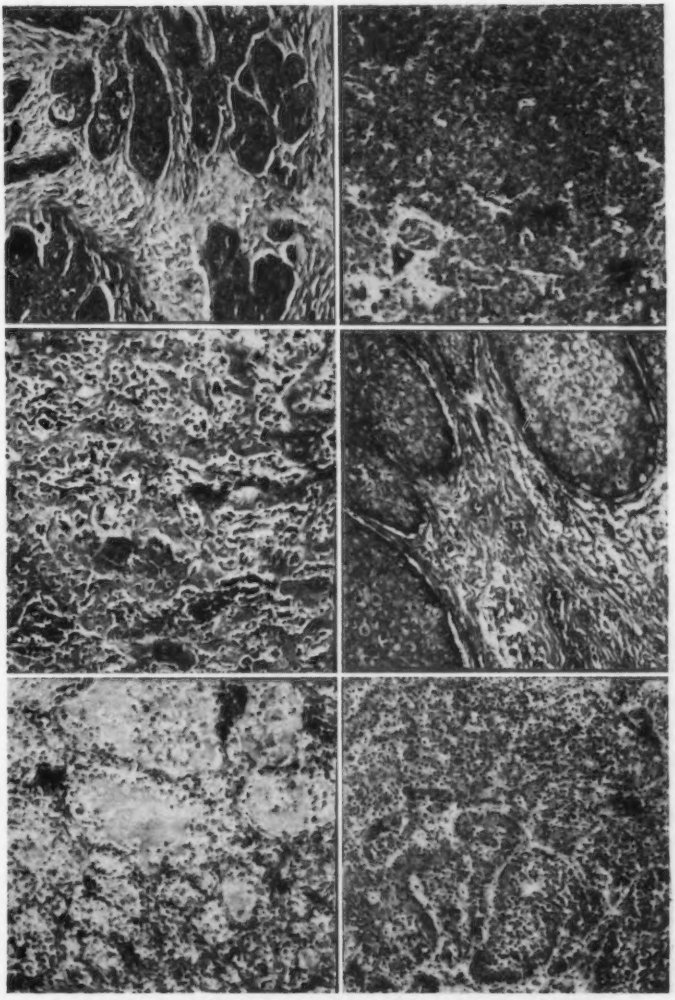

Fig. 1 (top left) Epithelioma. Pennant's parrakeet (× 120)
Fig. 2 (top right) Malignant hepatoma. Leadbeater's cockatoo (× 120)
Fig. 3. (middle left) Osteoma of the humerus. Cut-throat finch (× 120)
Fig. 4. (mildle right) Plantar papilloma. Glossy cowbird (× 120)
Fig. 5. (lower right) Adenocarcinoma of the thyroid. Budgerigar (× 120)
Fig. 6. (lower right) Ovarian carcinoma. Green-winged macaw (× 120)

changes may incite the formation of osseous proliferations. Multiple exostoses, an inherited deformity, is not a true neoplastic process but closely resemble osteomata. The liver of this bird revealed parenchymatal degeneration with a few necrotic foci. No neoplastic change was evident in this organ.

Glossy cowbird *(Molothrus bonariensis minimus)*

Both feet of this specimen were swollen. It had been in the managerie for only 10 days and weighed 43 grams. The left foot had a papillomatous growth with a cellulitis extending up the shaft of the leg. A pneumonic consolidation affected both lungs. Microscopic examination revealed the foot lesion to be a papilloma (fig. 4).

Budgerigar *(Melopsittacus undulatus)*

An enormous, bilateral enlargement of the thyroids was evident in this male bird. Degenerative tissue extended ventrally from the thyroid glands over the pericardium. No metastases were noted. Microsections revealed an adenocarcinoma of the thyroid (fig. 5). This glandular tumor is by no means rare in domestic animals, and commonly involves the thyroid gland, gastro-intestinal tract, mammary glands, prostate gland, kidneys, uterus, and the glandular elements of the respiratory system.[7] Its epithelial cells assume a gland-like arrangement. Goitre usually preceeds tumor genesis.

Green-winged macaw *(Ara chloroptera)*

A private owner submitted this specimen to the London Zoological Gardens. It weighed over two pounds and presented a gonadal growth. Microscopic studies revealed this lesion to be an ovarian carcinoma (fig. 6). The ovary is undoubtedly the most frequent site of origin of carcinoma of the chicken. This type of tumor has not been successfully transplanted as has the testicular teratoma of roosters.

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SUMMARY

Six exotic birds of the following species (Pennant's parrakeet, Leadbeater's cockatoo, cut-throat finch, glossy cowbird, burgerigar, and green-winged macaw, were examined by the authors. Neoplasms encountered in these birds were

examined grossly and histologically. The six birds were affected with the following neoplasms: Pennant's parrakeet, epithelioma, right tibia; Leadbeater's cockatoo, malignant hepatoma, liver; cut-throat finch, osteoma, right humerus; glossy cowbird, papilloma, left foot; budgerigar, adenocarcinoma, thyroid gland; green-winged macaw, carcinoma, ovary.

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SALMONELLA HEIDELBERG INFECTION AS A PROBLEM IN PULLORUM DISEASE CONTROL*

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INTERFERENCE by various Salmonella spp. with the pullorum agglutination test has been reported by Hinshaw and McNeil[4] who discussed the common antigenic components shared by various types of Salmonella as causes of cross-agglutination encountered in testing programs. Pomeroy and Fenstermacher[6,7] reported on the isolation of 23 Salmonella from paratyphoid infection in turkeys. Occurrence and distribution of 6387 paratyphoid cultures isolated from fowl from all sections of the country, many of which shared the IX and XII antigenic components, were reported by Edwards *et al.*[2]

Since January 1956, when *Salmonella heidelberg* was first isolated from a "pullorum reactor" submitted to this laboratory, this organism has been a problem in the Pullorum-Typhoid Eradication Program in Connecticut. At that time it was thought of as another Salmonella occasionally isolated from specimens submitted for examination. Since 1953 *S. typhimurium, S. gallinarum, S. enteritidis,* and *S. kentucky* have been isolated from "'pullorum reactors", *S. infantis, S. worthington, S. tennessee* from other avian specimens, and *S. cholera suis* from a mink. Dr. P. R. Edwards[3] commented that

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S. heidelberg had not been reported in this country until 2 or 3 years ago. He further stated that this organism has now been found in all sections of the country, in poultry and in outbreaks of salmonellosis in man. Records of the Connecticut State Department of Health in Hartford, indicate that human cases of salmonellosis due to *S. heidelberg* increased from 9 in 1955 to 35 in 1956.

ISOLATIONS OF *S. HEIDELBERG*

The first isolation of *S. heidelberg* was made in January 1956 from a reactor called for postmortem examination (Spec. No. A59903), showing typical *S. pullorum* agglutination in the finding test and retest. By the use of cultural methods, as recommended by Jungherr *et al.*,[5] non-pullorum, but typical Salmonella cultures were isolated from a caseous plug in the oviduct and from the ovaries. These cultures were typed and identified by the Connecticut State Department of Health in Hartford as *S. heidelberg*. Since then *S. heidelberg* has been isolated from 30 birds representing 11 flocks reacting positively to the standard 1:50 dilution pullorum agglutination test. With the exception of isolations made from a White Leghorn and a Broad White turkey, all avian isolations could be traced to White Rock (WR) breeding flocks, where all or part of the stock had originated from a single source. *S. heidelberg* was isolated from the ceca of a 6-day-old WR chick submitted for examination because of high (3 percent of 7000) mortality, which continued in the flock until the birds were 2 weeks old. Two, 2-week-old WR chicks from another flock were submitted because of a one percent mortality in a pen of 1500. Isolations of *S. heidelberg* were made from the spleen, gall bladder and intestine. Mortality in this pen continued until the birds were 10 weeks old. One other case of chick mortality from Canada, and four isolations from Maine breeding stock during the fall of 1956 and spring of 1957 according to U.S.D.A., A.R.S., N.P.I.P. reports of Salmonella isolations, have become known to this laboratory. An isolation was made from the liver of a ranch mink (Spec. No. A73759) which had been fed "cull" WR chicks as part of its diet, another, from the intestine of a

pet gray fox (Spec. No. A73781) which received eggnog as part of its diet. Presumptive evidence would indicate an avian infective source in these last two cases.

EXPERIMENTAL

Egg Transmission. To determine the degree of egg transmission of *S. heidelberg,* three pens of 12 WR birds each (11 hens and 1 cockerel) were selected. Birds in Pen 1 were inoculated intraperitoneally (i.p.) with 1 ml of a 24 hour broth culture of *S. heidelberg,* Pen 2 served as controls, and the birds in Pen 3 were inoculated with 1 ml of the same culture *per os* (p.o.). Immediately after inoculation, egg production in Pen 1 dropped from eight to two eggs per day. Egg production in Pen 2 remained at an average of five eggs per day. A drop from seven to five eggs per day resulted from the p.o. inoculation in Pen 3.

Eggs were collected daily at about 1:00 P.M. and identified by pens. No sanitary precautions were used in going from pen to pen while picking up the eggs. Eggs were not washed nor cleaned in any way before being pooled. All eggs from each pen were broken into and mixed in a Waring Blendor for approximately 10 seconds or until all yolks were ruptured and mixed. One ml from each pool was inoculated into each of 2 tubes containing 10 ml amounts of Selenite F broth. Broth tubes were incubated at 37 C for 24 hours and then streaked onto Salmonella-Shigella (SS) agar plates. Any suspicious colonies appearing on these plates were inoculated onto Triple-Sugar-Iron (TSI) agar slants; those showing Salmonella like reactions were submitted for final typing and identification to the Connecticut State Department of Health in Hartford. Of 55 egg pools cultured per pen, only one egg pool from Pen 1 gave an isolation of *S. heidelberg* (Spec. No. A73779). Chase and Wright[1] isolated *S. pullorum* from 61 of 1000 eggs cultured from 55 known positive pullorum reactors. Cultures of 1000 eggs from normal birds failed to give isolation of any type of Salmonella.

Serologic Findings. Preinoculation tests of all birds in all three pens were negative in a 1:25 serum dilution to *S.*

heidelberg phenolized antigen at 48 hours. Seven days postinoculation, all birds in Pen 1 had a titer of 4+ in the 1:100 dilution at 24 hours with one bird showing a 3+ reaction at 1:3200. Three birds in Pen 3 developed a titer of 2+ in 1:25 dilution at 24 hours. All control birds in Pen 2 remained negative to all tests during the entire course of the experiment. Tests with *S. heidelberg* and *S. pullorum* somatic antigens were conducted weekly from the 7th to the 9th week of the experiment. Two birds in Pen 1 showed a homologous titer of 2+ and 4+, respectively in the 1:100 dilution at 24 hours. These same birds failed to show any titer in the 1:25 dilution against *S. pullorum* antigen.

Comparative tests with *S. heidelberg* and *S. pullorum* antigens against serums from birds in Pens 1 and 3 showing reactions to either antigen, are presented in table I. Results obtained indicated that not all birds infected with *S. heidelberg* would be detected with the standard pullorum agglutination test. Seven of the 13 reacting birds showed no titer to *S. pullorum* antigen (1:25 dilution) but gave at least a 2+ reaction (1:25 dilution or higher) with *S. heidelberg* antigen. Prelim-

TABLE I

Comparative somatic titers for *S. pullorum* and *S. heidelberg*, 9 weeks postinoculation with *S. heidelberg*.

	Bird No.	S. pullorum	S. heidelberg
Penn 1 i. p.	1	0	50
	2	200	800
	3	0	100
	4	400	800
	5	200	400
	6	0	50
	7	800	800
	8	0	50
	9	50	400
	10	0	25
Pen 3 p. o.	1	0	400
	2	0	25
	3	50	25

O = no titer at 1:25.
Titers 2+ or more at 24 hours.
S. heidelberg (IV, V, XII, 1, 2, r), and
S. pullorum (IX, XII), 0.25% phenolized antigens.

inary antigenic studies on 16 strains of *S. heidelberg* seem to indicate the presence of both the XII_2 and XII_3 factors in varying degrees among the strains tested. Double testing of *S. heidelberg* infected flocks may be necessary for complete elimination of the organism from the stock.

Arithmetic mean serum titers, taken at weekly intervals, revealed a general decrease over a period of 8 weeks as shown in table II. Seven birds in Pen 1 showed a titer decrease, while three birds maintained their titers. Pen 3 birds showed the same tendency except that the levels were lower. The bird in Pen 3 from which *S. heidelberg* was isolated gave a variable titer over the 8 week period and showed no reaction at 1:25 dilution when the final test was made.

TABLE II

Arithmetic mean titers for 10 birds inoculated with *S. heidelberg*

		Weeks Bled Postinoculation							
		1	2	3	4	5	6	7	8
Pen 1	i.p.	1180	960	580	750	445	362	490	347
Pen 2	Controls	0	0	0	0	0	0	0	0
Pen 3	p.o.	15	97	50	70	37	35	32	45

Fecal Swabs. Weekly fecal swabs were taken from all birds from the 7th to the 9th week. Swabs were incubated in Selenite F broth at 37 C for 24 hours and then streaked on SS agar plates. Suspicious colonies were transferred to TSI agar slants and Salmonella-like reactions were checked and typed. *S. heidelberg* was isolated nine times from five different birds in Pen 1. No isolations were made from any bird in Pens 2 and 3.

Necropsy Bacteriologic Findings. Four birds, two in Pen 1 and two in Pen 3, died during the course of the experiment. Cultures of the gall bladder, intestinal contents, and ovaries were negative for *S. heidelberg* in all four birds. Nine weeks postinoculation, all birds in Pens 1 and 3 were killed and subjected to examination using the aforementioned cultural methods. No gross pathologic lesions were detected. *S. heidelberg* was isolated from four birds in Pen 1 and from one bird in

Pen 3. The control birds in Pen 2 were not cultured because of negative fecal swab and serum evidence. Bacteriologic findings of birds cultured and found positive are shown in table III. It is interesting to note that only two of the five positive birds yielded *S. heidelberg* from the ovary, generally considered the organ of choice in Salmonella isolation techniques. Three isolations were made from the gall bladder and four from the intestinal contents.

TABLE III

Postmortem bacteriologic isolations of *S. heidelberg*, 9 weeks post-inoculation.

		Primary Media					
		Tryptose Agar			Selenite F Broth		
	Bird No.	Pericardial Sac	Peritoneum	Oviduct	Gall Bladder	Ovary	Intestine
Pen 1 i.p.	1	—	—	—	—	—	+
	2	—	—	—	+	+	+
	3	—	—	—	—	+	+
	4	—	—	—	+	—	—
Pen 3 p.o.	4	—	—	—	+	—	—

SUMMARY

S. heidelberg was found to be a problem in the Connecticut Pullorum-Typhoid Eradication Program when 30 isolations were made from "pullorum reactors" and three isolations from chicks, all traceable to a single breeding stock. Isolations were also made from a ranch mink fed "cull" WR chicks and a gray fox. Egg transmission and fecal contamination in artificially infected birds were demonstrated. Rises in antibody level for *S. heidelberg* and *S. pullorum* were obtained following *per os* and intraperitoneal inoculation with *S. heidelberg*. Results indicated that infection with *S. heidelberg* causes cross reactions in the standard pullorum agglutination test.

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A STUDY ON THE CRITERIA FOR EVALUATING INFECTIOUS BRONCHITIS IMMUNITY*

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METHODS for evaluating infectious bronchitis disease immunity have often been a subject ofconsiderable conjecture. In the past, serum neutralization tests and respiratory symptoms following challenge have been employed by many investigators.[5,8,9,10,11] To compare and study the different means of evaluating infectious bronchitis immunity was the objective of this investigation.

MATERIALS AND METHODS

Seventy-eight Rhode Island Red chickens were separated

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into an equal control and vaccinated group. The control group was given the killed Newcastle Disease (ND) vaccine at 11 weeks of age and kept on a farm 1 mile from the vaccinated group.

The vaccinated group was given killed ND vaccine and infectious bronchitis (IB) virus simultaneously at 11 weeks of age. The killed ND vaccine (formalin-inactivated, aluminum hydroxide adsorbed) was given intramuscularly at the rate of 1 ml per bird. The IB virus (Van 8), was received from Dr. Van Roekel in 1946. Allanto-amnionic fluid from the 8th egg passage was pooled, diluted 1:10 with nutrient broth and given intranasally to the birds at the rate of 0.05 ml per bird. These birds exhibited respiratory symptoms two days following exposure. Three weeks after vaccination all birds were challenged. The challenge was administered by dropping 0.1 ml of 10^4 minimum embryo infective doses (MEID) of Van 8 IB virus intratracheally.

Blood was collected by cardiac puncture from four birds in each group prior to challenge and the first day after and from five birds each day thereafter for 2 days. The serums from these blood samples were collected aseptically and serum neutralization (SN) tests were conducted on each individual blood sample according to the procedure described by Fabricant.[3] The antigen used for the SN test was the Connecticut IB virus strain #66579 at the 251st serial egg passage. The titers of the antigens and of the serum were calculated according to the 50 percent end points of the method of Reed and Muench.[12]

At 24 hours after challenge and thereafter every day for 7 days and also on the tenth day, 5 birds from both the vaccinated and the control groups were checked for the presence of bronchial rales, gurgling sounds and coughing. The birds were then sacrificed and tracheal exudate and lungs were collected aseptically from each bird. During the first week after challenge tracheal tissues were collected and fixed for sectioning. At the same time, the lungs, tracheas and air sacs were examined for gross lesions. The presence of cloudiness and caseous material in the air sacs was considered positive. Congestion and consolidation in the lungs were considered positive. Tracheal lesions were graded according to the severity of the lesions.

In collecting materials for virus isolation, the tracheal linings were rubbed with a sterile cotton swab and the exudate collected was suspended in 3 ml of nutrient broth. It was then stored at –20 C until ready for use. Pieces of the lungs were also collected and mixed with nutrient broth at the ratio of 1:5. The lung was ground and the suspension centrifuged. The supernatant was stored at –20 C for virus isolation studies.

From the tracheal exudates and lungs, virus isolation trials were carried out. For each ml of the above material, 50 mg. of streptomycin in 0.5 ml broth was added to control contamination. Ten-day-old chicken embryos were inoculated into the allantoic sac with 0.15 ml of mixture. For each sample, five eggs were used. Three serial passages were made before the samples were discarded. The isolation of infectious bronchitis virus was based on the finding of curled, dwarfed and/or dead embryos.

Sections of the tracheas were fixed in Bouin's solution, and processed by the paraffin hematoxylin-eosin method. The histopathological lesions were classified as described by Jungherr.[6,7]

RESULTS

The serum neutralization indices (NI) of a total of 18 serum samples taken at 0, 1, 2 and 3 days after challenge (table I) had an arithmetic mean of 1.4 for the control and 2.8 for the vaccinates or for each 0.1 ml of undiluted serum, there were 25 neutralizing doses for the control and 631 for the vaccinates.

Respiratory symptoms after challenge were not observed in the vaccinates. In the controls, the symptoms were common from the 2nd through the 7th day after challenge (table 2). No gross pathological lesions of the air sacs and lungs were found in the controls or in the vaccinates. In the tracheas, the gross pathological lesions were pronounced in the controls and, except in three cases, all vaccinates were negative (table 2).

The IB virus was recovered from the tracheal exudate of the control birds 24 hours after challenge and through the seventh day. It was not recovered on the tenth day. The virus was not recovered from the tracheal exudate of the vaccinated

TABLE I

Serum neutralization indices.

Days After Challenge	Control	Vaccinates
0	2.1	3.8
	1.0	2.4
	1.0	2.0
	1.0	None
1	2.1	2.1
	1.5	3.3
	2.1	3.1
	1.0	3.3
2	1.7	3.3
	1.3	3.1
	1.6	2.3
	1.6	3.3
	1.6	2.1
3	2.1	3.6
	1.0	2.9
	1.1	2.9
	1.0	2.1
	2.1	2.6
Mean	1.4	2.8

birds except in three cases, which was on the 5th (two cases) and 6th (one case) day, after challenge (table 3). In the control lungs, the virus was isolated 48 hours after challenge, and isolations were successful for 7 days. In the vaccinated group, no virus was isolated from the lungs (table 3).

The histopathological study of the tracheal sections showed positive lesions in 33 of 34 control birds and one of the 27 vaccinated birds (table 4).

DISCUSSION

The arithmetic mean of the SN indices of the control birds was 1.4, or 13 neutralizing doses in 0.05 ml of undiluted serum. These data correspond to Cunningham's[2] finding that 99.7 percent of known negative birds would be expected to have a titer no higher than 36. The arithmetic mean of the SN indices of the vaccinated birds was 2.8 or 631 neutralizing doses in 0.1 ml of undiluted serum. According to Fabricant[3], 100 neutralization doses indicates positive evidence of previous infection with IB virus.

TABLE II

Results of clinical respiratory symptoms and the gross pathological lesions in the tracheas.

		Days after challenge							
		1	2	3	4	5	6	7	10
Control birds									
Bird number	1	O/—	R/+++	R/++	R/++	R/++	R/+	R/+	O/+
	2	O/++	R/+++	O/++	O/+	R/++	R/++	O/+	O/+
	3	R/++	O/++	R/++	O/+	R/+	R/+	O/+	O/+
	4	O/++	O/+	R/+	R/++	R/+	R/++	R/++	O/+
	5		R/+++	R/+	R/++	R/+	O/++	R/++	O/+
Vaccinated birds									
Bird number	1	O/—	O/+	O/—	O/—	O/—	O/—	O/—	O/—
	2	O/—	O/+	O/—	O/—	O/—	O/—	O/—	O/—
	3	O/—	O/—	O/—	O/—	O/—	O/—	O/—	O/—
	4	O/+	O/—	O/—	O/—	O/—	O/—	O/—	O/—
	5		O/—	O/—	O/—	O/—	O/—	O/—	O/—

The numerator represents clinical respiratory symptoms:
R = Presence of bronchial rales, gurgling sounds and coughing.
O = Absence of respiratory symptoms.
The denominator represents the gross pathological lesions in the tracheas:
— = No lesions.
+ = Congestion of epithelial linings.
++ = Congestion plus a slight amount of tracheal exudate.
+++ = Congestion plus a large amount of tracheal exudate.

In 17 cases, there were gross, pathological lesions of the trachea not accompanied by respiratory symptoms. This indicates that the absence of clinical symptoms following challenge, in some cases, may not give the true immunity status of the bird. However, when all the control birds with respiratory symptoms from the 2nd through the 7th day were compared with those of the vaccinated birds, a definite difference was found.

The gross pathological lesions in the tracheas of the vaccinated and control birds showed significant differences

TABLE III

Results of the infectious bronchitis virus isolation from tracheal exudate and lungs.

		Tracheal Exudate and Lung							
		Days After Challenge							
		1	2	3	4	5	6	7	10
Control birds									
Bird number	1	+/—	+/+	+/+	+/+	+/+	+/+	+/+	—/—
	2	+/—	+/+	+/—	+/+	+/+	+/+	+/+	—/—
	3	+/—	+/+	+/+	+/+	+/+	+/—	+/+	—/—
	4	—/+	+/—	+/+	+/+	+/+	+/+	+/—	—/—
	5		+/+	+/+	+/+	+/+	+/+	—/+	—/—
Vaccinated birds									
Bird number	1	—/—	—/—	—/—	—/—	+/—	—/—	—/—	—/—
	2	—/—	—/—	—/—	—/—	—/—	+/—	—/—	—/—
	3	—/—	—/—	—/—	—/—	—/—	—/—	—/—	—/—
	4	—/—	—/—	—/—	—/—	+/—	—/—	—/—	—/—
	5		—/—	—/—	—/—	—/—	—/—	—/—	—/—

The numerator represents virus isolation from tracheal exudate.
The denominator represents virus isolation from lungs.
+ = Infectious bronchitis virus recovered.
— = Infectious bronchitis virus not recovered.

throughout the experiment. During this investigation, no gross lesions were observed in the lungs and air sacs. This did not correspond to Cunningham's[1] findings where he found congestion and edema of the lungs and cloudiness of the air sac membranes with accumulations of cheesy material following IB infection.

In this investigation, the IB virus was recovered from the tracheal exudate and lungs for seven days after inoculation. On the 10th day, none of the five control birds yielded any virus. Fabricant and Levine[4] were able to isolate IB virus from tracheal swabs of two pooled samples of experimentally infected adult chickens for as long as four weeks after inoculation. It is significant that the IB virus was consistently isolated in the control group but not in the vaccinated group.

TABLE IV

Results of the histopathological study of the tracheal sections.

Days after challenge	Control		Vaccinates	
	No. of treacheas with lesions	No. of tracheas with no lesions	No. of tracheas with lesions	No. of tracheas with no lesions
1	4		1	2
2	4	1		3*
3	5			3*
4	5			3*
5	5			5
6	5			5
7	5			5

* Two tracheas were discarded due to loss of epithelial linings.

There was a significant difference between the number of virus recoveries from the tracheal exudate and lungs of the control and that of the vaccinated birds, and this method may serve as an effective means of evaluating IB immunity. However, the work connected with this method is tedious and expensive and in reality may not be very practical.

The histopathological lesions of tracheas following an infection by IB virus were described by Jungherr, *et al.*[6,7] This investigation demonstrated that immune birds (except in one case) had no tissue reaction when challenged with 0.1 ml of 10^4 MEID of the homologous virus.

This method, when properly done, provides a sensitive criterion in evaluating IB immunity, and also provides a permanent record of the investigation by preservation of a section of the trachea. In this study several tracheas had to be discarded due to loss of epithelial linings. This damage was done when the tracheas were rubbed with cotton swabs for virus isolation. Care should be taken to collect the tracheal tissue for sectioning prior to the collection of tracheal exudate.

SUMMARY

A study was conducted to compare the different means of evaluating infectious bronchitis immunity in a group of susceptible and immune birds. It was shown that when groups of 5 birds were studied on 7 consecutive days and also on the 10th day after challenge, any one of the following cri-

teria could be used satisfactorily: respiratory symptoms, gross pathological lesion of the trachea, histopathological lesion of the trachea, and recovery of the virus in the tracheal exudate and lungs. However, each testing procedure was of value only for a limited period and none were effective over the total sampling period.

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THE DISTRIBUTION OF THE INFECTIOUS SYNOVITIS AGENT IN THE TISSUES OF ARTIFICIALLY INFECTED CHICKENS*

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THE ETIOLOGICAL agent of infectious synovitis has been isolated by several workers. Wills[5] reported the presence of this agent in the renal, hepatic and splenic tissues as well as in the exudates of the hock and keel bursae. Bacteriological examination of these same tissues was made but no colonies were produced on nutrient agar, blood agar or PPLO agar (Difco). The agent did poduce mortality when inocula prepared from the above mentioned tissues were injected into the yolk sac of 7-day embryonated chicken eggs. This initial study and later reports by Wills,[6,7] first suggested the virus-like nature of the synovitis agent.

Olson[4] reported the isolation of the agent by inoculation of the synovial exudate which is characteristic of the disease into the yolk sac of 6-day embryonated chicken eggs Cover[1,2] *et al.* stated that the agent was isolated from inocula prepared from the liver, spleen and exudate of the synovial sacs by similar embryo passage. Filtration experiments using Seitz filters showed that the agent passed the 5.0 and 2.0 micron pads but was held by the 0.1 micron pad.

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Lecce[3] *et al.* cultured, stained and examined by the dark field microscope, synovial exudate and embryo passage material, but found no evidence of spirochetes, bacteria or pleuropneumonia-like organisms. Electron micrographs of crudely purified allanto-amniotic fluid showed cocco-bacilliform elements 0.2 to 0.5 microns in size. These structures appeared to have an external limiting membrane surrounding a protoplasmic body. From the above facts, Lecce suggested that the agent of infectious synovitis had properties most commensurate with a rickettsia or large virus.

Although the course, treatment and signs of the disease have been reported rather completely, the localization of the agent in the body tissues has not been definitely established. The purposes of the experiments to be described in this paper were (1) to ascertain which tissues contain the virus and (2) to establish the time of onset and persistence of the virus in the body of artificially infected chickens.

MATERIAL AND METHODS

All tissues or other specimens harvested for virus assay were taken from birds showing typical signs of infectious synovitis. Tissues were ground with sterile sand and nutrient broth in a mortar. Such tissues were diluted aproximately 1:5 on a weight volume basis. The fluid portion of such a mixture was poured into clean, sterile glass tubes for storage. Specimens of the intestine were washed in three changes of sterile saline or broth to remove the intestinal contents before grinding. Specimens of the intestinal contents, when used for virus assay, were first diluted 1:5 with sterile saline or nutrient broth and then filtered through a Seitz 2 micron pad. Such filtrates did not produce undesirable reactions when inoculated into chickens. However, on occasion, they were still too contaminated for embryo passage. In such instances 10,000 units of procaine penicillin G were added per ml. of sample to reduce embryo mortality caused by bacterial contaminants. In one experiment the saline used for washing the intestinal specimens was also used as an inoculum (duodenal washings).

The samples of bile were taken by syringe and needle prior to the extirpation of the gall bladder. The gall bladder specimens were rinsed with saline or nutrient broth before grinding. In order to obtain a sample of connective tissue, a section of the comb was used. In such cases the comb was disinfected with alcohol prior to harvesting. Blood samples were taken with a syringe and needle by cardiac puncture. The blood sample was allowed to clot and the serum removed after the clotted sample was held overnight in the refrigerator.

Once prepared as indicated above, all inocula were held at —15C until they were used. No inoculum was held longer than one week before passage to birds and embryonated chicken eggs. All birds used in the virus assay were either straight New Hampshires or Arbor Acre-Vantress crosses and were between 4 and 6 weeks of age. The portal of entry was either by intravenous or intramuscular inoculation. The dose used in all cases was 0.5 ml per bird. After inoculation, the birds were placed in isolation and observed daily for signs of the disease over a three-week period. In certain experiments, those birds which survived this period were sacrificed and examined for lesions. A sample was considered to be positive when it produced typical signs and gross pathologic changes in the test birds.

When the viral assay included passage in embryonated chicken eggs, the test was not considered negative until three serial passages failed to yield the agent. Five embryonated eggs were used in each test passage and each received a dose of 0.2 ml via the yolk sac. The eggs were candled daily and those which were found to be dead were examined immediately for lesions characteristically produced by the synovitis agent. If the tissue inoculum produced the typical pathologic changes of enlarged liver, splenomegaly, subcutaneous hemorrhages and congestion of the kidneys, or mortality or both, it was considered positive for the virus.

All chickens used as tissue donors for these tests were experimentally infected either by intravenous or intramuscular inoculation of the agent. All inocula were thoroughly checked in the laboratory for freedom from all but the infectious synovitis agent.

RESULTS

Experiment 1—The chicken used for tissue sampling in this experiment was infected by intravenous administration of 0.5 ml of chorio-allantoic fluid harvested from the third embryo passage of strain 294. The tissue specimens were harvested when this bird had well developed signs of the disease on the 10th day post inoculation. Inocula were prepared from the liver, blood serum, duodenum, contents of the duodenum and exudate from the flexor synovial sheath (leg). The virus assay was made by intravenous administration of 0.5 ml into each of five birds for each specimen.

Positive evidence of the virus was found in the inocula prepared from the blood serum, liver, duodenum and synovial exudate but not from intestinal contents.

Experiment 2.—The donor chicken in this case was infected by the same infective material and in the same manner as in experiment 1. The following specimens were harvested from this bird at the time the signs of the disease were very apparent: bile, liver, blood serum, spleen, cardiac muscle, duodenum, contents of the duodenum, kidney, pectoral muscle and synovial exudate from the flexor tendon sheath (leg). Five assay birds were inoculated intramuscularly with 0.5 ml of each specimen.

Positive results were observed in the birds which received the serum, liver, synovial exudate, cardiac muscle, kidney spleen and skeletal muscle specimens, but was not evident in birds injected with the duodenal contents, bile or duodenum.

Experiment 3.—There were three donor chickens used in this test. Each had previously received 0.5 ml intramuscularly of infective chorio-allantoic fluid harvested from the fourth embryo passage of strain 96. All birds had typical signs of the disease and the specimens were taken at random from them. The following specimens were used: trachea, mesentery, sciatic nerve, kidney, pancreas, comb, bile, jejunum, contents of the jejunum, liver, pectoral muscle, flexor tendons of the leg with their associated synovial sheaths, lung, cardiac muscle, air sac and testes. For the virus assay, each of three

chickens was given a 0.5 ml intravenous dose of each inoculum. This test was paralleled by an assay in embryonated chicken eggs.

Experiment 4.—As in experiment 3, three donor birds were used to obtain the tissue specimens. These birds were injected by intravenous inoculation of 0.25 ml of diluted synovial exudate obtained from the previous experiment. The following samples were taken: trachea, air sac, lung, rectum, flexor tendons and associated synovial sheaths (leg), gall bladder, duodenum, cardiac muscle, pectoral muscle, spleen, liver, testes, ovary, mesentery, pancreas, cecum, kidney, keel bursa, comb, sciatic nerve and ureter. Inocula were prepared and each was tested by inoculation of 0.5 ml intravenously into each of three birds. Embryonated chicken eggs were also used as a test for the presence of the virus.

Experiment 5.—This experiment was similar in almost all respects to experiment 4. Several additional specimens were taken from the infected birds. These were duodenal contents, duodenal washings, bile, synovial membranes from flexor digital sheath, contents of the rectum, and contents of the cecum. Each specimen was tested by intravenous administration into five birds and also was tested in embryonated chicken eggs.

The results from experiments 3, 4 and 5 can be given together. In general, all inocula tested were positive except the intestinal contents, bile, and the synovial membrane. Positive evidence was not found for every specimen on all three tests. Embryonated chicken eggs as an indicator for the presence of virus was less sensitive than birds. The complete results of these three experiments are found is table 1.

Experiment 6.—In the studies designed to determine the duration of viremia, the following procedure was followed. The culture used in this experiment was a composite prepared from liver, spleen, kidney and synovial exudate from several birds which had previously received 0.5 ml intravenously of chorio-allantoic fluid harvested from an embryo passage of strain 96. The tissues and exudate were ground with sterile sand and nutrient broth and was centrifuged at

TABLE I

Isolation of the agent of infectious synovitis from tissues of birds artificially infected

	Number of birds showing signs of infectious synovitis over no. inoculated			Results of embryo passage for evidence of the agent		
Experiment No.	3	4	5	3	4	5
Duodenum	—	1/3	5/5	—	neg.	pos.
Duodenal washings	—	—	5/5	—	—	neg.
Duodenal contents	—	—	0/5	—	—	neg.
Jejunum	0/3	—	—	neg.	—	—
Jejunal contents	0/3	—	—	neg.	—	—
Rectum	—	2/3	5/5	—	neg.	pos.
Contents of rectum	—	—	0/5	—	—	neg.
Cecum	—	0/3	5/5	—	neg.	neg.
Contents of cecum	—	—	0/5	—	—	neg.
Liver	3/3	3/3	5/5	pos.	neg.	pos.
Bile	0/3	—	0/5	neg.	—	neg.
Gall bladder	—	1/3	1/5	—	pos.	neg.
Pancreas	1/3	0/3	1/5	neg.	neg.	neg.
Spleen	—	2/3	4/5	—	neg.	neg.
Kidney	3/3	3/3	4/5	pos.	pos.	pos.
Ureter	—	1/3	1/5	—	neg.	neg.
Testes	3/3	—	—	pos.	—	—
Ovary	—	3/3	2/5	—	neg.	pos.
Trachea	3/3	0/3	0/5	pos.	neg.	neg.
Lung	3/3	3/3	5/5	pos.	pos.	neg.
Air sac	3/3	0/3	1/5	pos.	neg.	neg.
Cardiac muscle	3/3	2/3	—	pos.	neg.	—
Skeletal muscle (pectoral)	2/3	1/3	5/5	pos.	neg.	neg.
Keel bursa	—	3/3	5/5	—	pos.	pos.
Flexor tendon and sheath	0/3	1/3	1/5	neg.	pos.	pos.
Flexor synovial sheath	—	—	0/5	—	—	neg.
Mesentery	0/3	0/3	4/5	neg.	neg.	neg.
Nerve	3/3	2/3	0/5	neg.	neg.	neg.
Comb	1/3	0/3	0/5	pos.	neg.	neg.

— = no test made.

1000 rpm for 3 minutes to remove the large particles of tissue. The supernatant was stored at —15 C.

Twenty-four birds 5 weeks of age were inoculated intravenously with 0.5 ml of this inoculum and 26 birds received 0.5 ml intramuscularly. These birds were placed in isolation and observed daily. At 48-hour intervals following inoculation, two birds from each inoculated group were destroyed and samples of liver, spleen and blood were taken. The samples

from the two birds were pooled and inocula prepared as described in the previous experiments, except for the blood. The clot and serum were ground with sterile sand and nutrient broth (dilution 1:5). Three birds were inoculated intravenously with each of the inocula prepared from these specimens. Thus, three birds received the material from the liver tissue of the original intravenously inoculated birds and three received material prepared from liver harvested from the intramuscularly inoculated birds, etc. Tests for virus were made on the 2nd, 4th, 6th, 8th and 10th days.

In parallel to the bird inoculations, tests were conducted with the same inoculum by injecting five embryonated chicken eggs for each test.

Positive evidence of the virus was found in all inocula prepared. Thus the virus was present in the liver, spleen and blood from the first sampling at 48 hours post inoculation through the 10th day.

DISCUSSION

It appears from the above data that in birds artificially infected by intramuscular or intravenous administration, the infectious synovitis agent is present as a true viremia. The only specimens which consistently did not contain the virus were the various intestinal contents and bile. In two tests the jejunum was found to be negative. However, all other intestinal tissues were positive almost every time. It therefore seems probable that the jejunum also harbored the virus even though it failed to show its presence in these two tests. Not all tissues produced a positive test every time they were tested. This may have been due to variations in their preparation, enzymatic activity or dilution during the preparation of the inocula.

The presence of the virus in the blood and tissues 48 hours after exposure was to be expected from previous results. However, the duration of the viremia was surprising. Additional tests are being conducted to ascertain its maximum duration.

SUMMARY

The etiological agent of infectious synovitis was found

in almost all body tissues tested following intravenous or intramuscular inoculation of chickens. The agent was not found in the intestinal contents, in the bile or in the synovial membrane. By the methods used in these experiments a true viremia was produced 48 hours post inoculation and was still present on the 10th day post inoculation.

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THE INCREASED INCIDENCE OF VISCERAL LYMPHOMATOSIS IN BROILER AND REPLACEMENT BIRDS*

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IT IS generally conceded that the greatest amount of mortality due to visceral lymphomatosis (VL) in chickens occurs in young pullets soon after they are in heavy production.[2,5] The condition, however, may occur at any age after 4 weeks.[2] As early as 1949, there has been concern with the increasing incidence of VL in broiler age birds.[10] The great diversity of tissues which may be involved in VL has been well established. Although the presence of skin and muscle lesions have been reported, the impression is gained that such involvement is associated with prolonged cases. Jungherr[4] has described muscular lesions in pheasants which were suffering from neural lymphomatosis. Other authors[1,3,7,8,9] have reported the histopathologic alterations of muscular tissue induced by intramuscular injections of viable tumor cell suspensions.

The purpose of this paper is to report the occurrence of an unusually extensive involvement of VL in broiler age and young replacement birds in the Delmarva area. The case which received the most critical study and which is primarily reported here (Case 1) was referred to the Poultry Pathology Laboratory at the University of Delaware, Newark, Dela-

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ware in December 1956 by a Federal Poultry Inspector. The processing line under his surveillance had yielded an unusual number of condemnations due to VL for a period of several months, dating back principally to the summer of 1956. The presence of muscular lesions which were believed to be a part of the lymphomatosis involvement in many of the condemned carcasses was of considerable concern. The second case (Case 2) reported here was encountered in a replacement flock of 15,000 birds in which there was considerable alarm over the extreme mortality and high number of cull birds due to VL involvement at a very young age.

Case 1. The chickens involved in this case were a Vantress-Arbor Acre cross and were 9½ to 10 weeks of age when processed. These birds were raised and processed in a self-contained operation which included a number of rearing houses of considerable capacity and a processing plant which handled the entire production plus some birds grown off the premises. It was thus possible to determine facts relative to the condition insofar as the source of chicks, management procedures, etc. were concerned. The operation involved a rapid turn-over of birds so that each house was filled with chicks as soon as possible after the previous group was processed. A system of double brooding was practiced in which the house was filled to twice its normal capacity for the first four weeks. Then the flock was divided and raised in two houses. All other aspects of rearing the birds were similar to those observed in any large poultry operation, with the possible exception that a more rigorous sanitation program was followed. There appeared to be no unusual mortality in birds of those houses which had a high percentage of VL lesions on the processing line. The presence of a leg weakness and paralysis during the last two weeks of the growing stage was a common complaint in all houses.

The specimens examined were obtained as condemned carcasses from the processing line with the cooperation of the inspector in charge at varying intervals over a period of several months. Gross lesions were noted and tissues taken for histological examination. Tissue sections were prepared in the

usual manner and stained with hematoxylin-eosin. In addition, several attempts were made to select living birds in an effort to obtain material which could be used to produce the condition experimentally. This was somewhat difficult as there appeared to be little correlation between external appearance and internal lesions.

At one stage of our observations, a week's condemnations were evaluated. VL was responsible for from 20 to 34 percent of the daily condemnations. This compares with a national average of slightly over 8 percent of all condemned carcasses during the year of 1956.[6]

PATHOLOGY

The gross appearance of the pathologic changes in the internal organs were characteristic of VL. The liver, spleen, and ovaries were most consistently involved, although almost every organ was observed to be affected from time to time. The liver typically was enlarged with a variation of nodular and diffuse involvement. In some instances of the diffuse form, the liver had a gelatinous consistency and disintegrated easily under pressure. In some cases the spleen was enlarged three to four times its normal size and had a milky-white appearance. The ovary was hyperplastic, granular, and considerably enlarged in those instances where it was severely affected. All of the gross changes with the many variations seen in VL were present.

Histologically, there was an extreme infiltration by both mature and immature lymphocytic cells with the mature type usually predominating. In some cases, difficulty in establishing the structure of the organ was encountered as only masses of lymphocytes could be seen. This was particularly true of ovarian tissue in which only a few scattered follicles could be discerned. It should be pointed out that such lymphocytic infiltration was also seen in tissues which were not observed to be affected on gross inspection. In fact, histological changes associated with VL were seen eventually in every tissue examined.

Skin lesions were generally associated with the feather

follicle but not limited to this structure. In extreme cases, such lesions assumed an appearance of scabs with a brownish-crust formation. Typically, there were whitish distinct nodules which were quite easily detected, particularly in the dressed bird. Histologically, there was again an extreme accumulation of lymphocytic cells located primarily in the dermis and arranged in circular foci.

The muscular alterations were of particular interest especially in view of their prevalence. These lesions were observed in both the superficial and deep muscular layers and were most commonly found in the pectoral muscles. There was a variation in severity of the changes from narrow, barely discernible, whitish streaks in some carcasses to nodular formations elevated above the surface producing necrosis of the tissues. Distortion, which occasionally produced a "ridging effect" of the muscle, resulted as the tissue changes progressed. The areas so involved exhibited discoloration varying from a whitish gray with loss of normal luster to a definite yellowish-orange appearance. In the most severe and apparently the most protracted cases, the necrosis progressed to ulcer formation accompanied by gelatinous exudate. Some of the earlier changes were apparently accompanied by some hemorrhage into the area. Lesions seen in the thigh muscles were of a hemorrhagic and darkened nature but were rare in our observations. These muscular changes were present in conjunction with and in the absence of visceral changes and were also seen in well fleshed birds with no evidence of systemic involvement.

The histological changes observed in the muscular lesions were variable, as would be expected from the gross observations. In those specimens exhibiting only small, white streaks on gross examination, there was slight, lymphocytic infiltration between the muscle fibers with apparently little change in the muscle tissue proper. This was accompanied by hemorrhage in some cases. In more severe involvement, the lymphocytes produced a separation of the muscle bundles and fibers and occasionally resulted in a fragmentation of the fibers. The infiltration of the tissues apparently begins in the con-

nective tissue septa. In the tissues showing the greatest amount of gross change, there was complete destruction of muscular tissue and replacement by lymphocytes. Muscle fibers which were present in such areas exhibited fragmentation, homogenicity, and a loss of myofibrils. In a few sections, evidence of slight scar tissue formation was observed.

Case 2. The chickens involved in this case were a Nichols' 12 strain and were 8 weeks of age when the condition first became prominent. A complaint of excessive mortality and cull birds was given by the owner when specimens were submitted to a diagnostic laboratory. The history also included an outbreak of chronic respiratory disease which had occurred in part of the flock at four weeks of age which had been treated with antibiotics in the feed. The lesions observed at autopsy were primarily those of VL and included enlarged, grayish-white livers and spleens and consistently, enlarged, granular ovaries. Other organs were involved in different birds. While gross, lymphoid changes were noted in the peripheral nerves, these were not as pronounced as the changes in the visceral organs. Further observations of the flock, however, revealed signs of neural lymphomatosis, i. e., drooping wings, leg paralysis and pendulous crops. Cultures of liver and spleen on blood and MacConkey agar were negative for bacterial pathogens. Histological examination of tissues grossly involved revealed an extensive infiltration with lymphocytic cells particularly in the liver and ovary. The spleen had areas of lymphocytic accumulations and appeared to be hyperplastic.

As this flock was intended for replacement purposes, birds were examined periodically from the time they were first presented to the diagnostic laboratory until the birds had been on range for several weeks. They consistently had changes associated with VL. Losses of as high as 50 per day out of 15,000 birds were encountered during the age period from 8 to 12 weeks. Rigid culling was performed before the birds were moved to range, but losses continued to be excessive.

It would appear that VL and the neural form of lympho-

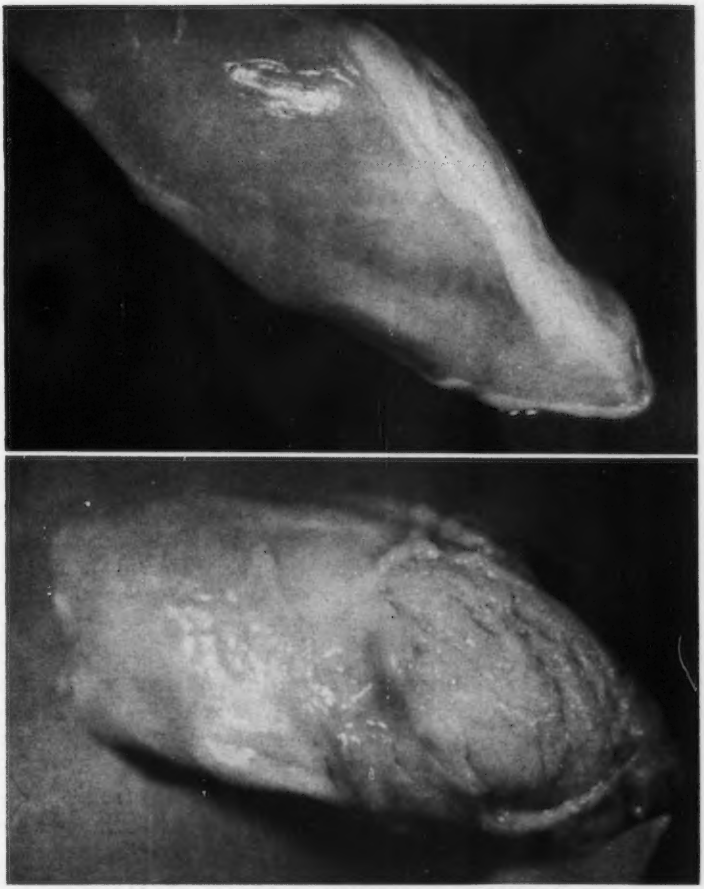

Fig. 1. (top) lymphocytic accumulation. (Case # 1)

Fig. 2. (bottom) Extreme lymphocytic infiltration with resulting necrosis in pectoral muscles. (Case #1)

matosis occurred concurrently in the flock, and as such, produced a higher loss than would generally be associated with either form at the particular age period. The regularity and severity with which the visceral involvement was observed, both in birds exhibiting signs of nerve involvement and in those not showing such signs, is of interest in view of the age at which the condition was noted.

DISCUSSION

The prevalence of the muscular lesions in Case 1 is of considerable significance as it represents another aspect of VL involvement responsible for the condemnation of carcasses. Such changes may escape detection unless the skin is slipped forward and the pectoral muscle examined. It is probable that as more poultry undergoes inspection more muscle involvement will be seen. The development of these lesions in such young birds may indicate that the VL agent has become more pathogenic, the birds more susceptible or both.

The definite visceral involvement noted in Case 2 is quite striking when the age of the birds affected along with the high losses encountered are considered. While considerable mortality and cull birds were seen in this case, muscular lesions were apparently absent. On the other hand, mortality was not a problem in Case 1.

The source of infection in both of the cases reported was difficult to establish. There was no contact with adult birds in either case and there appeared to be no correlation between the high incidence of condemnations due to VL and flock location during the early brooding period in Case 1. While it is quite possible that egg transmission from breeding flocks was the most important factor in these cases, there is no definite proof that it occurred.

From a diagnostic standpoint, the less severe muscular lesions may be confused on gross examination with similar appearing changes associated with monocytosis. Microscopically, the lymphocytic infiltration observed in lymphomatosis should be sufficient for differentiation. The hemorrhage which

may occasionally accompany the earlier stage of muscle infiltration may be mistaken for a lesion of the hemorrhagic syndrome. Again the typical histological changes of lymphomatosis along with the absence of hemorrhagic changes elsewhere in the body will serve as differential features.

SUMMARY

Two outbreaks of visceral lymphomatosis (VL) in young chickens which occurred during 1956 and 1957 in the Delmarva area are reported. The high incidence of VL in these cases suggests a possible increase in the pathogenicity of certain strains of VL for young birds, an increased susceptibility of the birds or both. The frequent occurrence of muscular lesions and the resulting condemnations on the processing line is unusual. Diagnostic problems concerning these changes are discussed briefly. The poultry industry is necessarily alarmed and concerned since this condition is apparently increasing in poultry flocks.

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THE INFLUENCE OF FURAZOLIDONE ON EXPERIMENTAL AND NATURAL PULLORUM INFECTION IN THE CHICKEN*

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FURAZOLIDONE (nf-180) has been investigated relative to its chemotherapeutic value concerning treatment and prevention of salmonellosis in poultry. Most of the studies in America have been done on fowl typhoid and paratyphoid, while studies with *Salmonella pullorum* have, for the most part, been undertaken in England.

Using day-old chicks, Smith[8] found that the 0.04 percent furazolidone level in the feed was more effective in controlling pullorum infection than were the 0.02 percent and the 0.01 percent levels. Early treatment and prolonged medication produced the most satisfactory results. The carrier incidence in a group of chickens was not entirely eliminated, but was reduced to a very low level. Similar results were obtained by Wilson[11] who did not observe a significant decline in titers and also isolated *S. pullorum* from some of the carriers.

Gordon and Tucker[4] reported that 11 of 13 treated adult carriers were found free of pullorum disease 34 to 84 days after initial treatment, and the blood titers dropped in 10 of the 13 treated birds. On the contrary, Wilson[12] demonstrated that the feeding of a 0.04 percent level of furazolidone in the mash continuously for 12 days failed in most cases to cause significant or permanent lowering of titers. After treatment, *Salmonella* persisted in 20 of 26 treated birds.

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In the treatment of *S. gallinarum* infection of turkey poults, Grumbles *et al.*[3] observed that the 0.011 percent level of furazolidone was more effective than the 0.0055 percent level in controlling mortality from the disease. However, when the treatment was discontinued, clinical manifestations of the disease reoccurred among the survivors of the exposed and medicated groups. Similar results were reported by Wilson.[11] The carrier incidence in chicks and adult birds has been investigated by Harbourne and Sellers,[5] Harbourne,[6] Lucas,[7] and Smith.[9] They showed that although furazolidone treatment was helpful in the control of fowl typhoid, some of the treated birds remained carriers.

Smith[10] and Wilson[11] also showed that the 0.04 percent level of furazolidone was more effective than the lower level against *S. typhi murium* infection in turkey poults and chicks. However, an appreciable number of carriers remained among the treated birds.

After preliminary trials, Cosgrove[2] recommended for treatment of fowl typhoid the 0.011 percent level of furazolidone in the feed for two weeks followed by a 0.0055 percent level until the birds are sold. This is in contrast to the English workers who recommended a 10-day curative treatment at the 0.04 percent level (Blount[1]).

The present investigation was undertaken to study the effect of furazolidone upon naturally and artificially pullorum-infected chickens of various ages.

MATERIALS AND METHODS

From a naturally infected breeding flock, 16 Rhode Island Red (RIR) hens were obtained to study the effect of furazolidone treatment upon the carrier state. Two trials were run using eight birds in each trial. Furazolidone* tablets were administered on the basis of 40 mg/kg body weight, and the average daily dose for 10 days was 105 mg per bird. This dosage approximated the 0.07 percent level in the feed.

* The furazolidone tablets were obtained through the courtesy of Hess and Clark, Inc., Ashland, Ohio.

In subsequent experiments, RIR chickens obtained from a pullorum-clean flock maintained at the University Experimental Farm were used. The birds were weighed, banded, and caged in pairs. Initial preinfection blood samples were collected to reaffirm the pullorum-free status.

In each trial, the chickens were divided into three groups and treated as follows: group 1 received furazolidone tablets for 10 days beginning at time of infection; group 2 received the tablets for 10 days beginning one week after infection; and group 3 was maintained as the unmedicated controls. The use of tablets assured each bird receiving a definite dosage, and as reported by Grumbles[3], this may be an important factor especially if the birds are off-feed.

The experimental design for the various trials is presented in table 1. The birds in trials 3 to 7 were infected *per os* with a 24-hour broth culture of *S. pullorum* by means of a glass syringe fitted with a blunt needle. Care was taken to avoid injury to the mucous membranes. The pullorum culture used in these trials was isolated originally from an active field case.

All birds were bled weekly and the sera were tested by the tube method in two-fold serial dilutions beginning with the 1:10 dilution. The tests were read after 24 hours incubation at 37 C and at room temperature overnight. In all trials either a 2+ reading in the 1:20 dilution or a 1+ reading in the 1:40 dilution was considered as the minimum criterion for a positive titer.

Bacteriological cultures were made at necropsy from the pericardium, liver, spleen, and peritoneum, on chicken infusion (CI) agar and inoculated into CI broth. The gall bladder contents were cultured in CI broth. Selenite broth was inoculated with ileal and cecal swabs. Ovaries were ground in a Waring blendor with CI broth and inoculated into selenite broth.

Following 24 to 48 hours incubation at 37 C, the broth cultures were plated on CI agar and on MacConkey's agar and incubated overnight. Typical and suspicious colonies of *S. pullorum* were selected for further examination. The original

TABLE I

Design of studies of the effect of furazolidone upon naturally and artificially pullorum infected chickens of various ages

Trial	Test group	Number of birds	Age	Sex	S. pullorum inoculum	Furazolidone medication Daily dosage, mg per bird	Duration of trial
1	1	8	17 months	F	Natural	Average dosage 105 mg	42 days
2	1	8	18 months	F	Natural	Average dosage 105 mg	56 days
3	1	4	4 months	F	1 dose of	70 mg	39 days
	2	4			1.0 ml	70 mg	
	Controls	2				—	
4	1	6	9 months	F	3 daily	100 mg	49 days
	2	6			doses of	100 mg	
	Controls	4			1.0 ml	—	
5	1	12	6 months	F	3 daily	50 mg	50 days
	2	12			doses of	50 mg	
	Controls	8			1.0 ml	—	
6	1	12	18 months	F	3 daily	60 mg	53 days
	2	12			doses of	60 mg	
	Controls	8			1.0 ml	—	
7	1	6	9 weeks	M	2 doses of	20 mg	45 days
	1a	6			0.5 ml 5	50 mg	
	2	6			days apart	20 mg	
	Controls	6				—	

F = females
M = males

agar plate cultures were examined after incubating 48 hours. The identification of the pullorum isolates was confirmed by standard bacteriological procedures.

RESULTS

The serologic, bacteriologic, and the gross pathologic results in the 7 trials are presented in table 2.

Trials 1 and 2. Sixteen naturally infected adult chickens were treated with furazolidone tablets for 10 days at an average daily dose of 105 mg per bird. The trials were terminated 42 and 56 days after initial treatment. All sera were positive for pullorum infection with the tube agglutination test. At the conclusion of trials 1 and 2, the serum titers showed the following distribution among the birds: five birds with 40, one with 80, six with 160, two with 320, and two with 640. There was little or no decline in the final titers when compared with the pretreatment titers. Any change was limited to one serial dilution except in three birds whose titers declined 2 dilutions. *Salmonella pullorum* was isolated from 12 of the 16 birds. Fourteen birds revealed typical ovarian changes as the prominent gross pathologic change. Wilson[12] observed that distorted ovules were the most common finding as the residual infection in carriers.

Trials 3, 4, 5, and 6. Although the furazolidone dosage varied, the results in these trials are somewhat similar and therefore will be combined. The dosage in tablets approximated the range level of 0.04 percent to 0.07 percent in the feed.

The results reveal that among the 34 chickens in group 1, seven were serologically positive and 12 birds manifested gross pathologic changes. The seven birds serologically positive at necropsy were first positive the third week after inoculation (these birds were in trials 4, 5, and 6). At necropsy the titers ranged from 320 to 1280. Recovery of *S. pullorum* was obtained in six birds which also manifested lesions. The titers of the remaining 27 birds were essentially negative throughout the trial and *S. pullorum* was not isolated.

In group 2, 19 of the 34 chickens were serologically posi-

TABLE II

Results of studies of the effect of furazolidone upon naturally and artificially infected chickens of various ages

Trial	Test group	Number birds	Number of birds with gross pathology					Total number of birds positive		
			Pericarditis	Hepatitis	Splenitis	Peritonitis	Oophoritis	Lesions	Serological	Bacteriological
1	—	8	2	1	1	3	8	8	8	8
2	—	8	0	1	0	1	6	6	8	4
3, 4	1	34	6	5	4	2	3	12	7	6
5, 6	2	34	7	10	7	8	9	15	19	12
	Control	22	12	12	9	8	9	18	22	20
7	1	6*	2	5	3	0	—	6	1	5
	1a	6*	2	2	2	0	—	4	1	1
	2	6*	1	1	0	0	—	1	3	4
	Control	6*	5	0	5	0	—	6	6	5

* Males

tive and 15 birds manifested gross pathologic changes. The serum agglutination titers developed rapidly since the furazolidone medication did not begin until one week after inoculation with the organism, and all birds showed positive titers by the second week. At the conclusion of the trials, the serum titers of these 19 birds had the following distribution: five birds with 40, three with 80, two with 160, three with 320, three with 1280, and three with 2560. Twelve of the 19 birds yielded *S. pullorum* on necropsy. Fifteen birds which were serologically negative at time of necropsy failed to yield *S. pullorum*. These birds had positive titers (40 to 640) by the second week after infection, and there was a gradual decline toward the termination of the experiment.

All 22 chickens in the control group were serologically positive and 18 manifested gross pathologic changes. All but one bird showed a strong positive titer by the second week after infection. *Salmonella pullorum* was isolated from 20 birds. The two birds which failed to yield the organism had low positive titers (40 and 160).

Trial 7. Nine-week-old cockerels were medicated with furazolidone tablets at 20 mg and 50 mg per bird. These dosage levels approximated 0.02 percent and 0.045 percent of the drug in the feed.

In group 1, only one of the six birds became serologically positive (titer of 80) by the fourth week after inoculation and remained positive for the duration of the trial. The failure to develop titers in these chickens may be due, in part, to the more resistant status of cockerels to pullorum infection. Six birds manifested gross pathologic lesions and five birds yielded *S. pullorum*.

Only one of the six birds in group 1a developed a positive titer (80) by the seventh or last week of the trial, but four birds manifested gross pathologic changes. *S. pullorum* was isolated from one bird. Since the dosage level is the only difference between groups 1 and 1a, the results suggest that the higher level of medication is more effective in controlling the infection. Similar results have been reported by Smith.[8]

In group 2, three of the six cockerels were serologically

positive at time of necropsy with titers of 320, 320, and 1280. Only one bird showed gross pathologic changes, but four yielded *S. pullorum* on necropsy.

All six control birds were serologically positive at necropsy (titers from 80 to 1280) and manifested gross lesions. *S. pullorum* was recovered from five birds.

DISCUSSION

Experimental furazolidone medication of chickens which were naturally and artificially infected with *S. pullorum* has revealed interesting results. In naturally infected adult chickens, the drug failed to alter the serum agglutination titers to any definite degree when observed for a period of 42 to 56 days post-treatment. This is significant from a practical disease eradication viewpoint when flocks are tested for pullorum disease. Furthermore, *S. pullorum* could readily be recovered from the serologically positive medicated birds. It appears evident that once the infection and antibody level are well established in a flock, furazolidone treatment fails to alter the infection status. It appeared that the pathologic changes were as marked as one usually observes in reactor birds that have not been medicated. These limited results suggest very strongly that adult chickens with well-established pullorum infection do not respond favorably to medication. However, medication might be justified if the elimination of the organism from the host could be reduced or entirely prevented. This aspect of the disease was not investigated.

In artificially infected chickens of various ages, furazolidone medication exerted a definite influence on the infectivity of the organism and the pathogenesis of the disease in the host. Likewise, a similar response was observed by the lack of agglutinin production in the majority of the treated chickens irrespective of their ages.

Birds treated initially at the time of infection responded more favorably than birds initially medicated 7 days after infection. This appears very significant and suggests that in an outbreak of the disease, furazolidone therapy would greatly retard the spread of the infection and reduce the establish-

ment of the disease in susceptible birds. These results support the therapeutic application of this drug in acute outbreaks of the disease and in infected flocks that are subjected to eradication of the infection by the macroscopic agglutination test. However, the therapeutic recommendations should be prescribed by someone who has a full knowledge of the disease and the flock in question.

A significant observation revealed that some medicated birds may develop into carriers but fail to react. Unquestionably, this would create a very objectionable problem for a testing program. While this observation was made concerning very young males, it is possible that in adult birds this serological problem might not arise. However, this needs urgent investigation in order that the desired information can be obtained for the testing agencies which may deal with medicated infected flocks.

These limited trials suggest that further chemotherapy investigations be continued in artificially and naturally infected chickens and also turkeys.

SUMMARY

The effectiveness of furazolidone medication was investigated in chickens of different ages artificially and naturally infected with *S. pullorum*.

In naturally infected adult chickens treated with furazolidone at the rate of 40 mg/kg of body weight, the drug failed to reduce the agglutination titers. Seventy-five percent of the birds were bacteriologically positive for *S. pullorum* at necropsy 6 and 8 weeks after the initial treatment.

In experimentally infected chickens, 9 weeks to 18 months in age, the furazolidone medication definitely reduced the incidence of infection which likewise influenced the antibody response to the organism.

The higher drug levels administered were more effective in preventing the establishment of infection.

The drug treatment administered during the early stages of the disease appeared to be more effective than when the medication was initiated several days post-infection.

These results suggest that furazolidone therapy may be

an effective aid in decreasing the spread of infection and in reducing the losses from pullorum disease. The application of furazolidone medication may have great practical value in the eradication of the disease in conjunction with the testing method. However, more information is needed to support this contention.

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FURTHER STUDIES ON PULLORUM DISEASE IN TURKEYS†

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A GREAT deal has been published regarding pullorum disease in chickens. The bulk of the literature on this subject has been steadily accumulating since its discovery at the turn of the century. Pullorum disease in turkeys, however, commenced to be set apart from that of chickens with the advent of the whole blood test. Within the last 15 years many articles have been written which have dealt exclusively with this disease in turkeys.

Hinshaw *et al.*,[8] compared the tube and whole blood agglutination tests and, with the antigens in use at that time, they found the whole blood test to be about one half as efficient at the tube test for the detection of pullorum disease in turkeys. Bushnell,[1] also claimed that for testing turkeys the stained antigen was less satisfactory. On the other hand, Gauger,[6] reported close agreement between K-formula stained antigen and the tube-test antigen when testing experimentally and naturally infected turkeys which gave a complete reaction at 1/25 dilution or higher.

About the same time Corpron *et al.*,[3] using experimentally infected turkeys, found K-formula antigen little better than one-half as effective as the tube-test antigen for the detection

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of birds with a low titer or in the early and late stages of infection.

Two years later Garland *et al.*,[4] reported on comparisons of the tube test at 1/25 dilution, K-formula stained antigen, and Redigen. They found that the tube test and Redigen compared favorably while the K-formula antigen was the least sensitive for the detection of pullorum carriers in turkey flocks. Carpenter *et al.*,[2] using two groups of turkey poults, one infected at 1 day of age and the other at 14 days of age, found that the K-formula antigen was satisfactory only for a short time after infection and during egg laying periods. Gwatkin and Ozenis,[7] reported a 10 percent disagreement between whole blood and tube methods of testing. They favoured the tube test for the detection of pullorum disease in turkeys.

The fact that *S. pullorum* is shed from the ovary was reported by Gauger,[5] who reported 4.68 percent infected eggs laid. This was substantiated by Carpenter *et al.*[2] who recovered the infection from 6.7 percent of the eggs laid by an experimentally infected flock. In the latter case, infection had been established 29 weeks prior to egg production.

In the course of their studies, various workers have reported also on the fluctuating titers encountered with pullorum disease in turkeys. Corporon *et al.*[3] reported maximum titers on the ninth day after infection by both parenteral and oral routes. Carpenter *et al.*[2] and Gwatkin and Ozenis[7] found similar antibody peaks directly after infection which dropped in many cases to below a 1/25 titer within 8 weeks. They both found that the antibody titer rose again during egg laying periods but with succeeding clutches the peak titer was progressively lower. The latter report also noted adult transmission during the course of the experiment.

Most workers who conducted lengthy experiments agreed that a large percentage of turkeys which survived the initial infection eventually became negative to the test and they failed to isolate *S. pullorum* upon necropsy. However, the fact still remains that recovery is not always certain and in many cases does not occur before mating with the result that infected eggs may be laid.

Since the important period of transmission of pullorum disease in turkeys appears to be during the poult stage, we should continue to do our utmost to ensure disease-free breeding stock. With this in mind and since *S. pullorum* has reportedly been isolated from birds that have passed one or more negative tests, we undertook the task of reviewing *S. pullorum* detection in turkeys under conditions of natural and artificial infection.

EXPERIMENTAL METHODS

Both naturally and experimentally infected turkeys were included in this study. The naturally infected flock consisted of 204 birds. They were tested twice with a three week interval between tests. Infection in the flock was proven by isolation of *S. pullorum* from reactor birds.

Since the tube agglutination test employed at a 1/25 diagnostic titer frequently reveals a number of low-grade reactions that are not evident by other methods of testing, it was decided to investigate the carrier state of such reactors. About 950 sera were tested by the serum-plate method using Redigen and K-formula antigens. The sera were selected as a result of low grade reactions in the tube test. More than 100 eggs and dead-in-shell embryos were cultured from flocks possessing only low grade reactor birds. These eggs were removed at various times during the incubation period and all stages were represented.

In order to study further the type of reaction obtained with known *S. pullorum*, 30 turkeys 7 weeks of age were inoculated with 0.1 ml of *S. pullorum* by the intravenous route. Blood testing by whole blood and tube methods at weekly intervals commenced 10 days later. After 13 weeks of testing, serum-plate tests were done with the same antigens used for the whole-blood test. Ten normal turkeys of similar age and breeding were maintained separately. They were tested in a similar manner for 18 weeks at which time they were mixed with the inoculated group. Death and selections for necropsy throughout the experiment reduced the number in the pen to 23 at 40 weeks after inoculation. During the experi-

ment, all dead birds, regardless of the apparent cause of death, were cultured for *S. pullorum*.

All the eggs laid, approximately 360, by the infected group were either examined bacteriologically or incubated. Twenty poults from this group were raised to 12 weeks of age before necropsy and bacterial examination.

This experiment was repeated with 24 birds inoculated at 5 weeks of age. They were blood tested at weekly intervals for 35 weeks after inoculation. All birds were necropsied at the end of the experiment and examined bacteriologically.

Throughout these experiments, the antigens used were those authorized for approval in Canada, i.e. polyvalent tube and K-formula whole-blood antigens, and in addition Redigen (Columbus Vaccine Co.) was also used. Bacterial examinations of eggs, embryos, and birds for *S. pullorum* were conducted with aseptic precaution. Conventional methods and media were used for all procedures of isolation and identification of micro-organisms. Each organ was individually pulped in a Waring blendor and cultured. Three aliquots of each pulp were made into tetrathionate broth. The remainder was incubated overnight and cultured again the following day in a similar manner. Selective culturing was continued on MacConkey's agar medium.

Concurrent with this work, all sera reacting to the tube test in our routine testing laboratory were again tested by serum-plate method. Most often both Redigen and K-formula antigens were used. This procedure has been continued into the second testing season.

RESULTS AND DISCUSSION

The results of comparative serological tests conducted on a naturally infected flock of 204 turkeys were conflicting. There were 27 reactors to the polyvalent tube test, three to Redigen whole-blood test, none to K-formula whole-blood test, and 18 to the serum-plate tests with both Redigen and K-formula antigens. The Redigen was agglutinated better and faster than the K-formula antigen. The retest revealed four common reactors to the tube test and serum-plate tests but no reactors to the whole-blood tests. The Redigen was again

agglutinated more distinctly than the K-formula antigen. *S.* pullorum was recovered from all four of the reactors to the retest. This suggests, as others have, that the whole-blood test is not reliable for the detection of pullorum disease in turkeys.

Many low-grade reactions to the tube test were negative to other tests. Of the 950 sera which gave low grade agglutination in the tube test at a titer of 1/25 and originating from flocks with no history of pullorum disease, 140 reacted to Redigen and 62 agglutinated the K-formula antigen. The reactions with sera and whole-blood antigens were comparatively slow, fine and of a weak nature. They were not complete even though the reaction time was extended. The majority of these reactions did not go beyond the 1+ value on the plate test. These reactions were very different from the reactions seen when pullorum immune sera are tested by this method.

No *S. pullorum* was recovered from over 100 eggs and dead-in-shell embryos originating in flocks yielding only low-grade reactions. This suggests, but by no means proves, that such birds are not reacting as a consequence of the specific pullorum immunity of natural infection. It is indicated also that since no transmission of *S. pullorum* through the egg was detected in this group, that the serum-plate test might give more reliable results than the tube test in such instances.

During the experiment with artifically infected birds, *S. pullorum* was isolated at 2, 3, 6, 7 and 35 weeks after inoculation but was not isolated from any of the survivors beyond 35 weeks after infection. No *S. pullorum* was recovered from any of approximately 360 eggs cultured. Twenty poults from the infected stock reared to twelve weeks of age failed to yield *S. pullorum* on necropsy and bacterial culture.

The serological reactions observed when the artifically infected birds were tested were quite intense for a period of 8 to 10 weeks directly following infection. This intensity of reaction faded continually to the end of the experimental testing period of 35 weeks. In agreement with others this was affected by individuals and also by mating and laying periods. As the serological reactions became weaker, differences between testing methods became apparent. At 11 weeks after

infection there were no reactors to either whole blood test but 90, 85, and 60 percent of the 20 inoculated survivors were positive to the tube, Redigen serum-plate and K antigen serum-plate tests respectively. At 25 weeks after inoculation, these figures were 65, 7, and 7 percent of 16 inoculated survivors. By 35 weeks only a single bird remained as a fairly consistent reactor to the tube test and the serum-plate tests. From these tests it would seem that the tube test with polyvalent antigen was more sensitive than either the whole blood or the serum-plate tests. Whether a test as sensitive as this is required for the elimination of pullorum disease from breeding turkey flocks is a matter of conjecture at this time. These data did indicate that a serum-plate test could be of benefit to screen reactions obtained by the tube test.

Throughout the latter half of the experiment or during the chronic stage of the infection, titers seldom were above 2+ in 1/100 serum-antigen mixture. Many of the reactions were positive at 1/25 but negative at 1/50. Such sera frequently gave a reaction on serum-plate test of less than 1+.

Throughout the experiment there was no adult transmission of pullorum disease to negative controls. Since these birds were penned together from 18 weeks after the infection to the end of the experiment, it showed that under these conditions, pullorum transmission among adults during the chronic period of the disease is unlikely.

The results obtained when all reactions to the polyvalent tube test were checked by serum-plate method suggested the latter test to be more indicative of infection. For obvious reasons this conclusion has to be based on field results rather than bacterial isolation. More than 100,000 routine blood samples were involved with 17 infected flocks detected in 1956 and four infected flocks in 1957. Further support for this method is evidenced by the lack of outbreaks of pullorum disease in poults in Ontario during the past year.

SUMMARY

1. Under the conditions of these experiments, the whole-

blood test was not reliable for the detection of *S. pullorum* in turkeys beyond 8 to 10 weeks after infection.

2. In some instances, the serum-plate test with polyvalent antigens appeared to be more reliable for the detection of pullorum infection in adult turkeys than the tube test.
3. *S. pullorum* was not isolated from artificially inoculated turkeys beyond 35 weeks after infection.

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ABSTRACTS OF PAPERS PRESENTED AT THE 29th NORTHEASTERN CONFERENCE ON AVIAN DISEASES (FORMERLY ANNUAL MEETING OF THE NORTHEASTERN CONFERENCE OF LABORATORY WORKERS IN PULLORUM DISEASE CONTROL) JUNE 24-26, 1957, PENNSYLVANIA STATE UNIVERSITY, UNIVERSITY PARK, PA.

THE INCREASED PATHOGENICITY OF VISCERAL LYMPHOMATOSIS

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Complete manuscript published elsewhere in this issue.

ORAL THERAPY FOR ERYSIPELAS IN TURKEYS

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S. HEIDELBERG INFECTION AS A PROBLEM IN PULLORUM DISEASE CONTROL

W. E. Burr, M. Tourtellotte, R. E. Luginbuhl and E. L. Jungherr
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A STUDY OF THE CRITERIA IN EVALUATING INFECTIOUS BRONCHITIS IMMUNITY

P. W. Chang, A. H. Dardiri, Dorothy E. Fry and Vance J. Yates
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FLUORESCENT ANTIBODY TECHNIQUE IN VETERINARY RESEARCH

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The fluorescein labeled antibody technique as developed by Coons and his associates has provided an extremely valuable histochemical tool for study of the immunologic aspects of disease. This method, essentially, consists of conjugation of fluorescein isocyanate with gamma

globulin precipitated from the specific antiserum of the antigen to be studied. The isocyanate forms a durable bond with the immune globulin, and the resulting solution is used as a reagent (the conjugate) on frozen sections cut in a cryostat. Specific staining results when antibody antigen linkage takes place and is visible by means of fluorescence microscopy. Methods to insure specificity are employed. The method has been used by Coons and others to elucidate the distribution of antigen of many viral and other diseases and for basic studies in the localization of antibody formation.

In the veterinary field, fluorescent antibody was first applied to infectious canine hepatitis, and the inclusion body was shown to contain viral antigen. The method was useful in the study of the pathogenicity of the disease. In canine distemper, fluorescent antibody has been useful in proving the virus nature of the inclusion body and for detailed studies of the pathogenicity. It also is being employed for the diagnosis of distemper in living animals. Moulton has published a study of encephalitis of canine distemper by this method. Studies of the details of the pathogenicity of the disease will be presented with slides.

Undoubtedly fluorescent antibody technique will prove an invaluable aid in many phases of veterinary research.

THE DISTRIBUTION OF INFECTIOUS SYNOVITIS AGENT IN THE TISSUES OF ARTIFICIALLY INFECTED CHICKENS

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THE RESPONSE TO AVIAN PPLO VACCINATION I. LABORATORY TRIALS

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Pleuropneumonia-like organisms (PPLO) are always associated with chronic respiratory disease of chickens. Data have been reported which indicate that vaccination or infection of chickens and turkeys with PPLO confers some degree of immunity. The causative agents of bovine pleuropneumonia and agalacita of sheep and goats confer a state of solid immunity on the host. In view of these facts, it seems desirable to review the feasibility of avian PPLO vaccination.

Young chicks were vaccinated by injecting 0.1 or 0.2 ml. of medium or egg propagated virulent PPLO (Winchester strain) into the pectoral muscle, beneath the skin of the neck or into the muscles of the lower leg. After periods of time ranging from four to nine weeks, groups of the chicks were challenged with different dilutions of homologous medium or egg propagated PPLO via the posterior thoracic air sac. One week after challenge the birds were necropsied and the air sac lesions were rated on a four plus scale according to their severity.

The results of these experiments indicate that under the conditions of the experiment, intramuscular or subcutaneous vaccination with live virulent PPLO does not produce grossly recognizable lesions; and, that vaccinated birds are somewhat refractory to subsequent challenge via the air sac route. A less severe method of challenge might reveal a greater percentage of birds to be refractory to challenge. A limited number of field trials should be conducted to determine the degree of refractivity of vaccinated birds to field infection.

The technical assistance of Mrs. Barbara Ann Browning, M.T./-ASCP, is gratefully acknowledged.

STUDIES ON CHRONIC RESPIRATORY DISEASE. I. HISTORY AND CULTIVATION OF BACTERIAL L-FORMS

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The history of true PPLO, including their nature and morphology, media and cultural methods, serology and pathogenicity are discussed. Using these as a basis, the similarity between the PPLO and L-forms of various bacteria are pointed out. These include such things as media necessary for growth and morphology of colonial growth. Definite differences exist, however, and these are illustrated and discussed. The demonstration of L-forms from various bacteria is reviewed and the bacteria involved are listed. L-forms may be induced by a variety of substances and the possibility of their occurrence in nature is considered. A detailed outline for the detection and cultivation of bacterial L-forms and the determination of the bacteria from which they are derived is presented.

VACCINES AGAINST *ESCHERICHIA COLI* INFECTION IN CHICKENS

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There are three types of *Escherichia coli* which are most commonly associated with the pericarditis complication of chronic respiratory disease. These have been identified by the Communicable Disease Laboratory of the U. S. Public Health Service. Type B belongs to "O" group 78 and has an "A" or capsular antigen. Type C belongs to "O" group 2 and has an "L" or envelope surface antigen and type D belongs to "O" group 1 and also has an "L" surface antigen. Serum from birds which have recovered from infection with any of the above types will give protection as will recovery from the disease itself or exposure to aerosols containing the organisms. *E. coli* can be removed from the body of chickens with large doses of streptomycin given intramuscularly. When infection is removed 24 hours after air sac inoculation the birds are protected from reinfection. If streptomycin is given 12 hours or less after air sac inoculation, no protection results. All of these methods are obviously unsatisfactory. A satisfactory vaccine may be prepared against type B by employing boiled or beta propriolactone or formalin killed organisms. Against type C only a beta propriolactone killed vaccine of one isolate was satisfactory. No satisfactory vaccine has been prepared against type D.

STUDIES ON CHRONIC RESPIRATORY DISEASE. II. THE REVERSION OF SO-CALLED "PPLO" TO BACTERIAL L-FORMS

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The fact that organisms having the characteristics of PPLO can be isolated from a majority of CRD cases has led to the belief that PPLO is connected with the etiology of CRD. Evidence is presented that the so-called "PPLO" isolated from CRD cases are actually L-forms of bacteria.

Twenty strains of so-called PPLO obtained from stations along the eastern coast (representing 6 states) and from one western state were studied. When inoculated into broth medium containing horse serum but no bacterial inhibitors, all 20 of these strains reverted to bacteria. Several transfers in broth may be necessary. In our experi-

ence all 20 strains reverted to bacteria by the 3rd transfer.

All 20 of the bacterial strains were gram + bacteria. The following genera were represented: corynebacterium, 1 strain; streptoccocus, 2 strains; and micrococcus (staphylococcus), 17 strains.

The data on these experiments indicate that true PPLO was not represented in these 20 strains. All were in fact L-forms of bacteria.

It is believed that the cultural techniques used to diagnose CRD are responsible for the appearance of these L-forms. Explanation of this postulation is presented.

CASE REPORT-SUSPECTED RELATIONSHIP BETWEEN EPIDEMIC TREMORS AND LENS OPACITY

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A PROCEDURE FOR THE DIFFERENTIATION OF SALMONELLA AND ARIZONA GROUPS FROM OTHER ENTERIC ORGANISMS

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Salmonella species and the Arizona group of organisms are the principal enterobacterial pathogens of poultry. In an attempt to simplify the detection of these organisms, lactose urea agar was developed by Sieburth (J. Bacteriol., *72*, 278-279, 1956). Although this medium permits simultaneous detection of lactose fermenters and organisms with urease activity, organisms which do not attack these substrates such as *Alcaligenes fecalis* and Pseudomonas species are not differentiated from Salmonella species and the Arizona group. In order to overcome this objection and permit further differentiation, mannitol iron semi-solid agar was developed to supplement lactose urea agar.

Mannitol iron semi-solid agar consists of bacto peptone (Difco) 15 grams proteose peptone No. 3, 5 grams mannitol 10 g, ferrous sulfate 0.2 grams, sodium thiosulfate 0.3 grams, phenol red 0.04 grams (or 20 ml of 0.2 per cent phenol red stock solution, Sieburth, 1956), bacto agar 3.0 grams, distilled water 1,000 ml and normal sodium hydroxide to pH 7.0 (approx. 3.6 ml). Heat about one third of the water to boiling, dissolve dry ingredients, bring up to volume, adjust pH, tube in 8 ml amounts, and autoclave for 15 minutes. An acid reaction indicates mannitol fermentation, surface bubbles indicate gas formation, growth away from stab indicates motility and blackening indicates hydrogen sulfide production.

The procedure used is to pick lactose non-fermenting colonies from selective plate media and to inoculate tubes of both lactose urea agar and mannitol semi-solid agar with the same colony. Organisms which alter the reaction of lactose urea agar or fail to ferment mannitol are discarded. The lactose urea agar slants of the remaining isolates are used to perform slide agglutination tests with polyvalent Salmonella and Arizona antisera, and to inoculate carbohydrate broths with the agglutinating isolates.

The technical assistance of Mrs. Nancy P. Oakey is gratefully acknowledged.

THE INFLUENCE OF FURAZOLIDONE ON EXPERIMENTAL AND NATURAL PULLORUM INFECTION IN THE CHICKEN

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OBSERVATIONS ON THE BONE STRUCTURE OF CAGED LAYING HENS

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Available reports of "cage layer fatigue" have usually failed to describe the pathology encountered. These clinical studies indicate that the condition is an osteodystrophy which most probably should be classified as osteoporosis. The tibial ash weights are sharply reduced and the percentage ash values are also subnormal. Judging from random samples of clinically normal caged layers, it appeared that mineral depletion of the skeletal structure was quite general in caged layers. Individuals that became lame frequently did so as a result of bone fractures and probably represent extremes or accidental cases rather than isolated abnormal birds in a flock. Blood Ca or P levels did not appear to offer a diagnostic aid due to great physiological fluctuation in these blood components related to egg shell formation. Some birds apparently paused in egg production as a result of mineral depletion of the skeletal system without developing lameness. Interruption of production appeared to allow sufficient restoration of the skeletal system to allow another period of egg production. No significant hepatic changes were found in affected specimens. The caged laying flocks studied were on rations generally considered adequate in respect to Ca, P and vitamin D levels.

Layers maintained on the floor on diets deficient in Ca and P developed skeletal abnormalities which could not be distinguished from affected caged layers.

CASE REPORT—RENAL COCCIDIOSIS IN GEESE

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A young Emden gosling markedly emaciated was presented for autopsy. No evidence of diarrhea was present. The most noticeable pathologic change was pale, streaked, and greatly swollen kidneys. The heart's blood, liver, and kidneys were cultured without recovery of any bacterial organisms. Microscopic sections of the kidneys were prepared and large numbers of coccidia were found.

The microscopic sections showed all parts of both kidneys involved. Large clumps of coccidia were found in the cortex adjacent to the capsule and throughout the kidney tissue. Much of the kidney tissue was destroyed. Few glomeruli were found, and those appeared compressed as though inactive but with large capular spaces.

Tubules filled with coccidia were found, many of which were in developmental stages. Much of the tubular epithelium was destroyed and clumps of developmental stages of coccidia were found in the kidney tissue. A cellular reaction was evident with many neutrophiles and eosinophiles present.

FURTHER STUDIES ON PULLORUM DISEASE IN TURKEYS

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Adler, H. E. 19, 235
Anderson, D. L. 349
Anderson, G. W. 338
Angstrom, C. I. 52

Baker, Norman F. 227
Bankowski, R. A. 195
Basch, H. I. 121, 349
Benton, W. J. 54, 312, 320
Berg, J. 19
Bletner, Ruth 82
Boyer, Clyde I. Jr. 42, 275
Brown, J. A. 42, 275
Burr, W. E. 298

Calnek, B. W. 208
Chang, P. W. 115, 304
Chomiak, T. W. 118
Chute, H. L. 67
Coffin, David L. 345
Cordy, D. R. 235
Cover, M. S. 52, 312, 320

Dardiri, A. H. 115. 304
Domermuth, Charles H. 346
Dougherty, E. III. 127, 130

Epps, N. A. 338

Fabricant, Julius 62, 101, 256
Fahey, J. E. 117
Fredrickson, T. N. 67
Fry, Dorothy E. 115, 304

Gentry, R. F. 75, 347
Gill, E. 94
Gross, W. B. 36, 347

Hill, R. W. 195
Hinshaw, William R. 1
Heddleston, K. 94
Hill, W. C. Osman 290
Hofstad, M. S. 165, 170

Johnson, E. P. 122
Jungherr, Erwin 118, 298

Kelton, W. H. 347
Levine, P. P. 101, 110, 136, 208, 256
Luginbuhl, R. E. 118, 298

Moore, E. N. 27
Munro, D. A. 82

Olesiuk, Olga M. 119
Olson, N. O. 82
O'Meara, D. C. 67, 91

Peckham, M. C. 247

Raggi, L. G. 195, 227
C. G. Rickard 256
Reynolds, I. 121
Rickard, Charles G. 130

Saunders, L. Z. 27
Seadale, E. H. 120, 122
Sevoian, M. 121, 136, 349
Sieburth, J. McN. 122, 180, 348
Shelton, D. C. 82
Smithcors, J. F. 18
Smyser, C. F. Jr. 328
Snoeyenbos, G. H. 121, 349
Somer, A. 94
Stubbs, E. L. 349
Sullivan, J. F. 94

Taylor, J. R. E. 101
Tourtellote, M. 298
Truscott, R. B. 338

VanRoekel, H. 119, 328

Wadsworth, James R. 290
Wichmann, R. W. 222
Williams, J. E. 105
Winterfield, R. W. 120, 122
Wright, M. L. 338

Yates, Vance J. 304
Yamamoto, R. 19

Artifact resembling focal necrosis in the livers of slaughtered White Pekin ducks, An (Dougherty and Rickard) 130
Beaudette, Fred Robert (Hinshaw) 1
Bone structure of caged laying hens, Observations on the (Snoenbos, Sevoian, Anderson and Basch) 349
Chorioretinitis, granulomatous, Blindness in turkeys due to (Saunders and Moore) 27
Chronic respiratory disease agent, Egg transmission of the—Preliminary report (Olesiuk and Van Roekel) 119
Chronic respiratory disease, Laboratory analysis of several field outbreaks of (Sullivan, Gill, Somer, and Heddleston) 94
Chronic respiratory disease, Studies on, 1. History and cultivation of bacterial L forms (Gentry and Kelton) 347
Chronic respiratory disease, Studies on II. The revision of so-called "PPLO" and bacterial L forms (Kelton and Gentry) 347
Coccidiosis, renal in geese, Case report 349
Contaminants in poultry live virus vaccines of egg embryo origin, Bacterial (Williams) 105
Differentiation of salmonella and Arizona groups from other enteric organisms, A procedure for (Sieburth) 348
Duck virus hepatitis, The pathology of (Fabricant, Rickard and Levine) 256
Epidemic tremors, Lens opacities in fowls possibly associated with, Case report (Peckham) 247
Erysipelas in turkeys, Oral therapy for (Boyer and Brown) 275
Erysipelas in turkeys, Studies on (Boyer and Brown) 42
Escherichia coli infection in chickens, Vaccines against (Gross) 347
Escherichia coli infection of the chicken eye (Gross) 36
Fluorescent antibody technique in veterinary research (Coffin) 345
Furazolidone on experimental and natural pullorum infection in the chicken, The influence of (Smyser and Van Roekel) 328
Furazolidone, The effect of, on the cultural and serological response of *Salmonella typhi-murium* infected chickens (Sieburth) 180
Hemagglutination-inhibition test, A simplified method for the titration of antigen in the beta (Gentry) 75
Hematology of broiler flocks, Preliminary investigations on (Fredrickson, Chute and O'Meara) 67
History collection, The Beaudette veterinary (Smithcors) 18
Immunity study of three types of Newcastle vaccine (abstract) (Dardiri, Chang and Fry) 115
Infectious bronchitis, Effects of, on the reproductive tracts, egg production, and egg quality of laying chickens (Sevoian and Levine) 136
Infectious bronchitis immunity, A study on the criteria for evaluating (Chang, Dardiri, Fry and Yates) 304
Infectious bronchitis virus, Dosage response of tracheal mucosa to (Jungherr, Chomiak and Luginbuhl) 118
Infectious sinusitis in turkeys, A serological study of (Hofstad) 170
Infectious sinusitis of turkeys, Egg transmission of (Hofstad) 165
Infectious synovitis agent, in the tissues of artificially infected chickens, The distribution of the (Cover and Benton) 312
Isolation of viruses from the respiratory tract of chickens, A modified chicken embryo inoculation technique for (Fabricant) 62
L forms, History and cultivation of bacterial, Studies on chronic respiratory disease 1. (Gentry and Kelton) 347
Lens opacities in fowls possibly associated with epidemic tremors, Case report (Peckham) 247

Neoplasms in exotic birds, Selected (Wadsworth and Hill) 290
Newcastle disease (B-1) and infectious bronchitis viruses, Response of eight-week old susceptible chickens to (Bankowski, Hill and Raggi) 195
Newcastle disease vaccine, Some factors influencing the immune response from administration of, through drinking water under laboratory and field conditions (Winterfield and Seadale) 122
P^{32} and S^{35} in bronchitis infected embryos, The distribution of (Levine) 110
Pathology of duck virus hepatitis, The (Fabricant, Rickard, Levine) 256
Paratyphoid outbreak in a poultry in a poultry breeding flock, A case report (Angstrom) 52
Pleuropneumonia-like organisms in chickens, Studies on experimental egg transmission of (Calnek and Levine) 208
Pleuropneumonia-like organism of chronic respiratory disease from tracheal exudate, A comparision of four *in vitro* methods for the isolation of the (Taylor, Fabricant and Levine) 101
Pleuropneumonia-like organisms of avian origin, Strain differences of (Adler, Yamamoto, and Berg) 19
Pleuropneumonia-like organism, The pathogenesis of the encephalitis in turkey poults produced by a neurotropic (Cordy and Adler) 235
PPLO infection in chukar partridges (Alectoris Graeca) Case report (Wichmann) 222
PPLO vaccination, The response to avian, 1. Laboratory trials (Domermuth) 346
Poisoning, Thiophosphate, in White Pekin ducks (Dougherty) 127
Pullorum disease in turkeys, Further studies on (Wright, Anderson, Epps and Truscott) 328
Pullorum infection in the chicken. The influence of furazolidone on experimental and natural (Smyser and Van Roekel) 328
Reversion of so-called "PPLO" and bacterial forms, The Studies on chronic respiratory disease II, (Kelton and Gentry) 347
Salmonella and Arizona groups, A procedure for the differentiation of, from other enteric organisms (Sieburth) 348
Salmonella heidelberg infection as problem in pullorum disease control (Burr, Tourtellote, Luginbuhl and Jungherr) 298
Salmonella typhi-murium infection in chicks, Observations on stress factors and serological response in (Sieburth and Johnson) 122
Serum neutralization test to indicate the relative live virus content of infectious bronchitis vaccine, A double (O'Meara) 91
Synovitis caused by the infectious synovitis agent, pleuropneumonia-like organisms and a combination of the two agents, Preliminary blood studies in chickens with a (Olson, Shelton, Munro, and Bletner) 82
Tetrameres americana (Cram, 1927) infection in domestic pigeons (Raggi and Baker) 227
Tissue culture studies of avian viruses (Fahey) 117
Tracheal mucosa, Dosage response of, to infectious bronchitis virus (Jungherr, Chomiak and Luginbuhl) 118
Vaccines against *Eschirichia coli* infection in chickens (Gross) 347
Viral agent, from chickens showing respiratory distress, The isolation of (Cover and Benton) 54
Visceral lymphomatosis in broiler and replacement birds, The increased incidence of (Benton and Cover) 320

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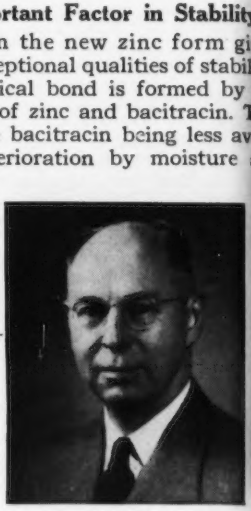

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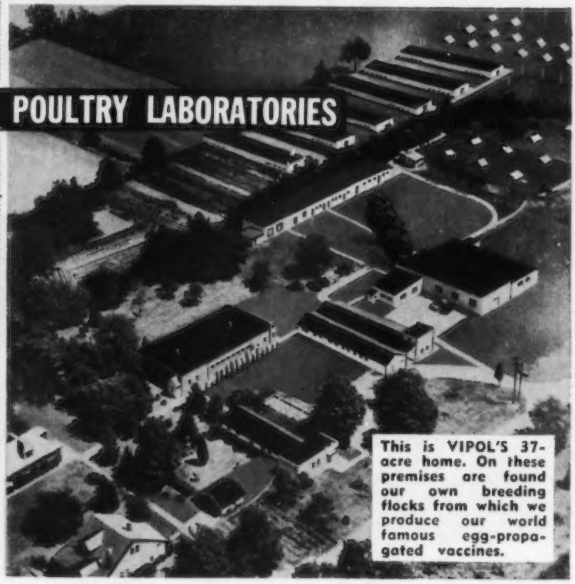

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